



# The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

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# STUDIES ON HYPOCHROMIC ANEMIA IN DOGS. I. THE EVALUATION OF A STANDARD BREAD DIET AND OF A MEAT DIET ON FORMATION OF HEMOGLOBIN BEFORE AND AFTER GASTRECTOMY<sup>1</sup>

By FREDERICK KELLOGG,<sup>2</sup> STACY R. METTIER AND KATHERINE PURVIANCE

(From the Division of Medicine, University of California Medical School, San Francisco)

(Received for publication January 25, 1936)

The almost constant finding of achlorhydria or hypochlorhydria in patients with idiopathic hypochromic anemia is now well known. Recent studies (1) of patients with this disease indicate that this abnormality may bear a definite relationship to the genesis of the anemia. It was shown that, in the treatment of patients with achlorhydria and hypochromic anemia, by the daily administration of a diet rich in food-iron, satisfactory formation of hemoglobin occurred only when the meal fed had been previously digested in vitro with hydrochloric acid and pepsin. This experiment seems of greater significance when it is emphasized that, in some of the patients, the anemia recurred after the predigested meals were discontinued. From these studies it was concluded that in chronic idiopathic hypochromic anemia, gastric dysfunction leads to a failure in the utilization of organic (dietary) iron, and that the disease is due to the resulting deficiency of iron.

Patients with hypochromic anemia comprise a group composed almost exclusively of females many of whom have subsisted on diets deficient in iron-containing foods and whose bodily stores of iron may be influenced by prolonged profuse menses. It seemed apparent, therefore, that these factors might alter the concept of achlorhydria as the sole etiologic agent. Accordingly, it was thought desirable to undertake further studies (2) using a laboratory animal in which these various factors could be more suitably controlled.

Hypochromic anemia has been reported to occur in man following gastrectomy (3, 4, 5). Ivy, Morgan and Farrell (6) have reported the occasional occurrence of a spontaneous anemia in gastrectomized dogs, and have also noted an increased tendency toward anemia in gastrectomized dogs

during pregnancy. These authors concluded that the removal of the stomach reduced the factor of safety in the dog, and that the additional strain of pregnancy was sufficient to produce an anemia. Gutzeit (7), Aron and Bauer (8), and Maison and Ivy (9), in their studies on the rat and dog, found that a similar anemia occurred following gastrectomy. Mullenix, Dragstedt and Bradley (10) reported that their dogs after gastrectomy showed a reduced capacity to form hemoglobin. These authors, however, failed actually to compare production of hemoglobin before and after operation. Other investigators (6, 11) reported that no anemia occurred in their laboratory animals following gastrectomy alone. The discrepancy between these reports suggests that not all dogs under these experimental conditions tend to develop a hypochromic anemia. The iron reserve of the animal and the demands for increased production of hemoglobin are factors which might influence the development of such a state.

Hypochromic anemia has been induced in the dog by Whipple and Robschelt-Robbins (12, 13) following bleedings repeated at frequent intervals. This anemia is an iron-poor anemia, and resembles the anemia in man associated with achlorhydria, gastro-intestinal disturbances, inadequate diet and chronic blood-loss.

The production of an artificial achylia gastrica in the dog by gastrectomy and of an hypochromic anemia by repeated bleeding should, therefore, under controlled conditions, offer a satisfactory means of determining the relationship between diet, digestion and hemoglobin production. An investigation, accordingly, was undertaken to determine the output of hemoglobin in dogs before and after gastrectomy.

## METHODS

Four healthy, adult, mongrel dogs were placed on the standard bread diet of Whipple and Robschelt-Robbins

<sup>1</sup> Supported by a grant from the Christine Breon Fund for Medical Research.

<sup>2</sup> Research Fellow of the American College of Physicians.

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Analysis of the gastric secretion following the oral administration of 20 cc. of 7 per cent alcohol showed the presence of free hydrochloric acid in each of the dogs prior to gastrectomy. After

recovery from the operative procedure, the upper intestinal content showed an absence of free hydrochloric acid even after the subcutaneous administration of histamine.

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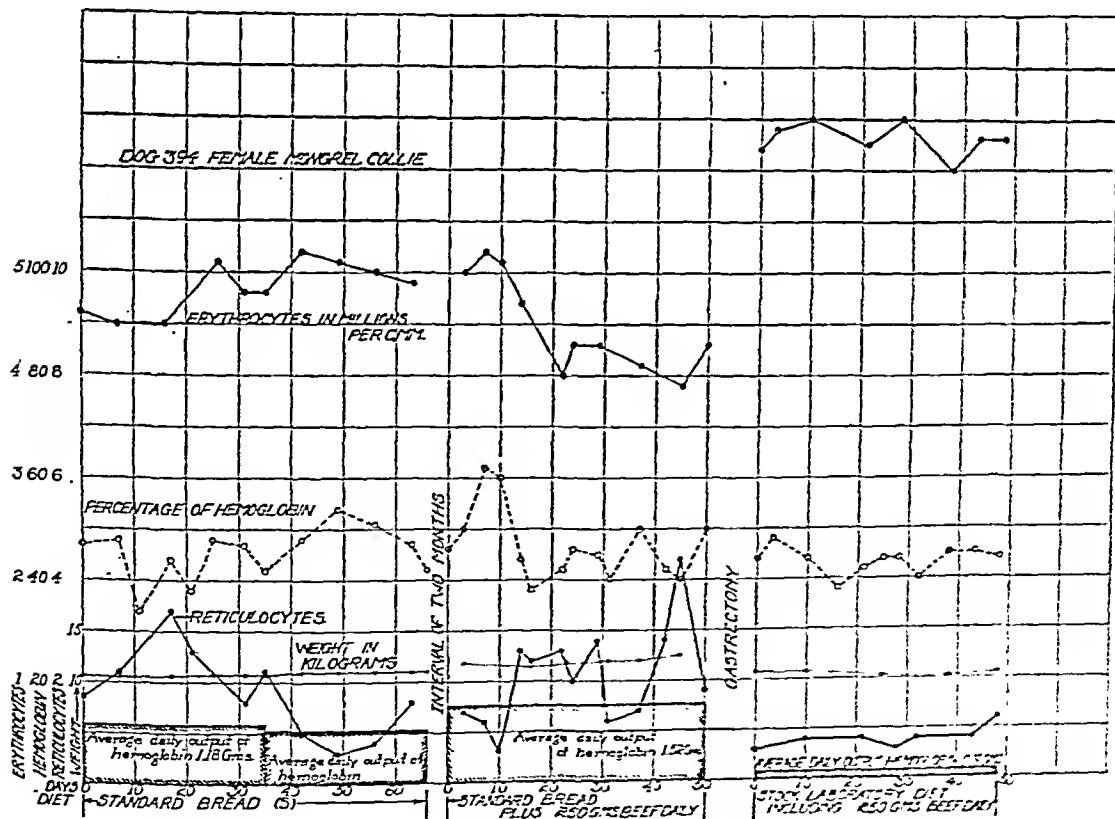


FIG. 2. COMPARISON OF HEMOGLOBIN OUTPUT BEFORE AND AFTER GASTRECTOMY.

Note the rise in erythrocytes following operation.



(12). This bread consists mainly of cereals, supplemented by salmon, tomatoes, yeast and cod-liver oil. Whipple states that this bread is a complete diet for an adult dog, and will maintain the animal in health for long periods of time. Furthermore, this ration is poor in factors favorable for the production of hemoglobin, the average output being close to 1 to 3 grams of hemoglobin per week, over and above the maintenance factor. An analysis of our bread showed an average of 2.6 mgm. of iron per 100 grams of dried bread.

The dogs were bled from the jugular vein sufficiently and at such intervals as to maintain the hemoglobin at a level from between 6 and 7 grams per 100 cc. of blood. A quantitative estimation of the hemoglobin removed was accurately made. The amounts removed represented the maximal production capacity of the bone marrow during the various experimental periods. By bleeding, an anemia was maintained until the production of hemoglobin became stabilized, for a period of from 3 to 8 months, depending on the individual animal. Presumably, at the end of this time the reserve stores of hemoglobin were exhausted.

Examinations of the blood were made by one person, using unvarying technique, throughout the experiments. Specimens of blood were removed at approximately the same hour twice weekly. Blood volume and determinations of hemoglobin, erythrocyte, reticulocyte and leukocyte counts were made as a routine procedure. Blood

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All dogs were given a vermifuge prior to the period of investigation, and stools were examined at monthly intervals to determine the presence of parasites and ova.

The technique of gastrectomy<sup>3</sup> performed upon these

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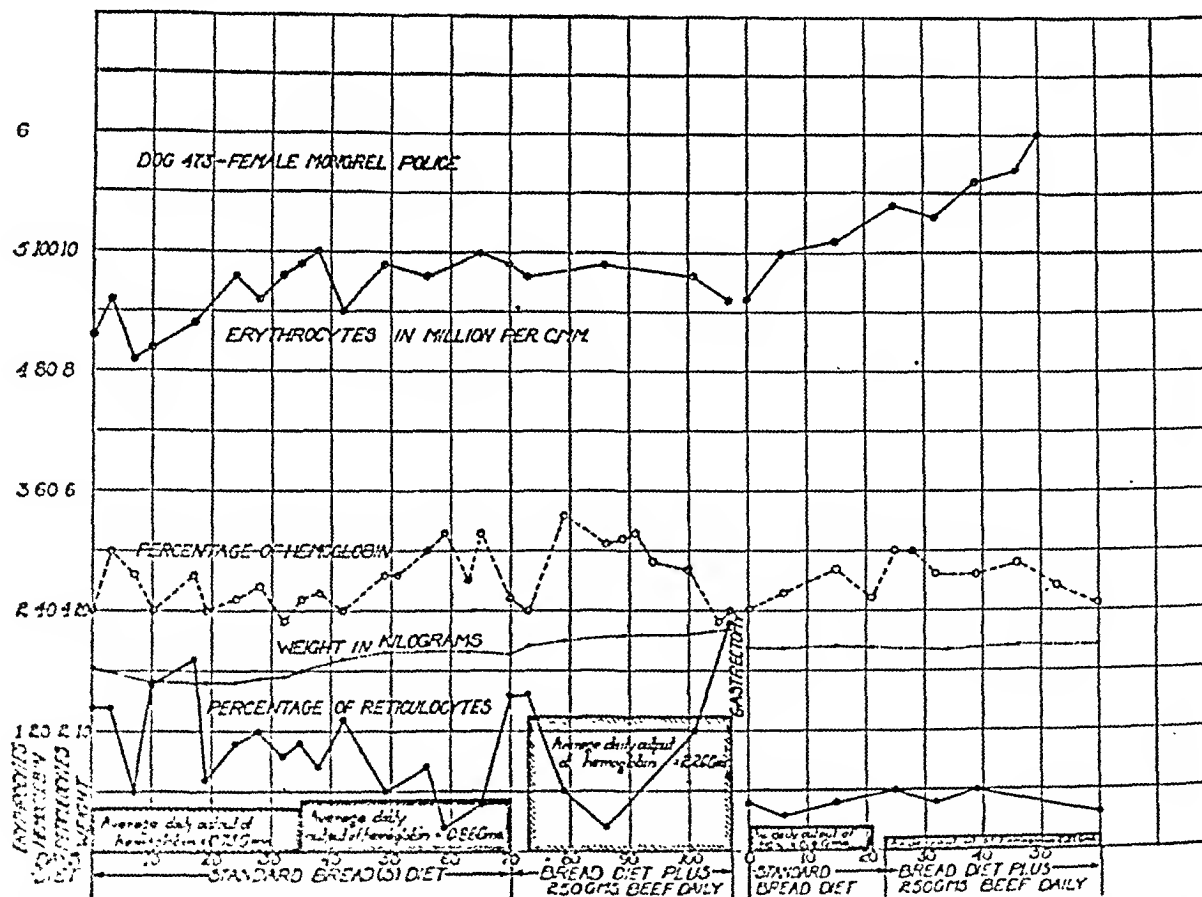


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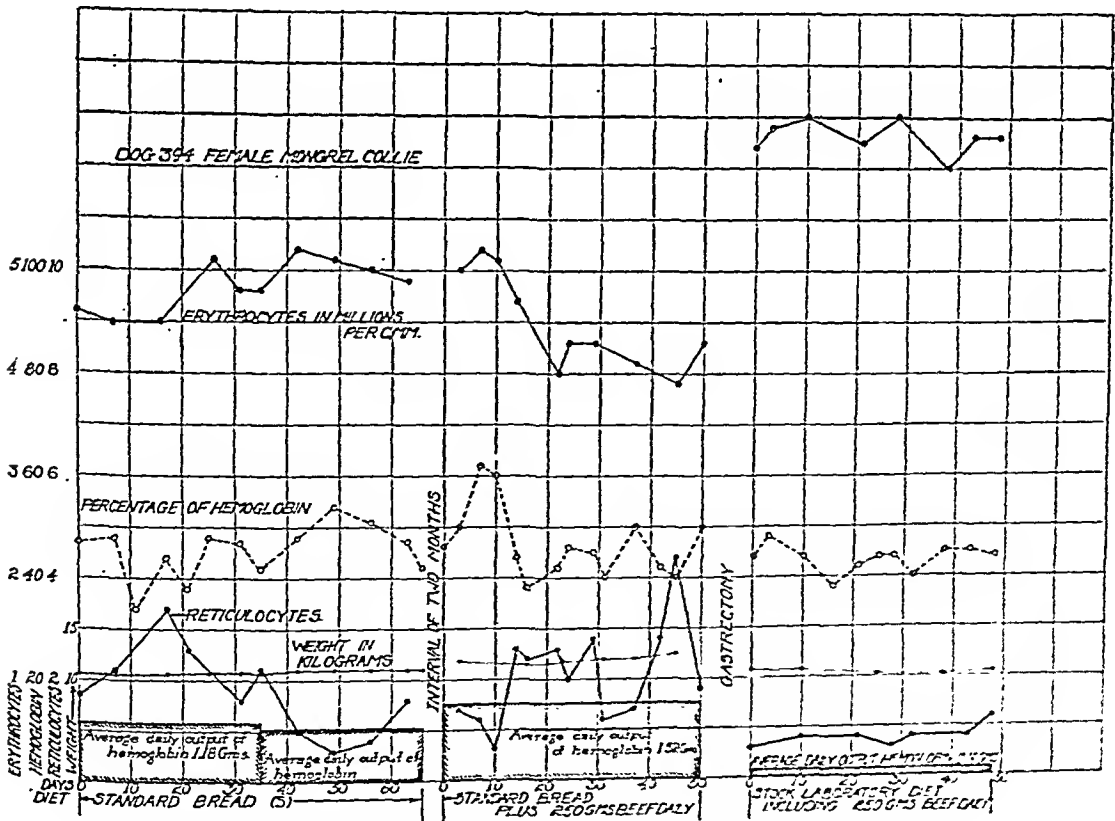


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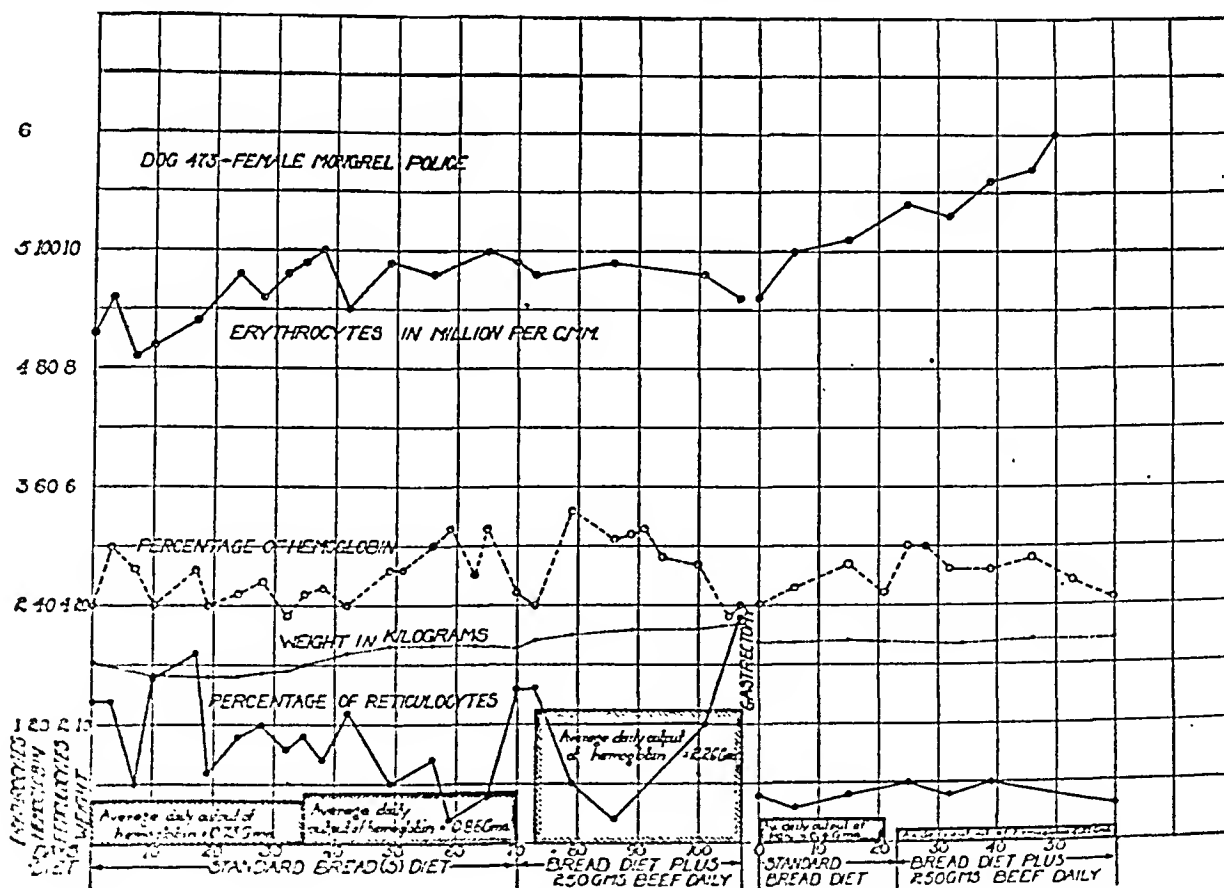


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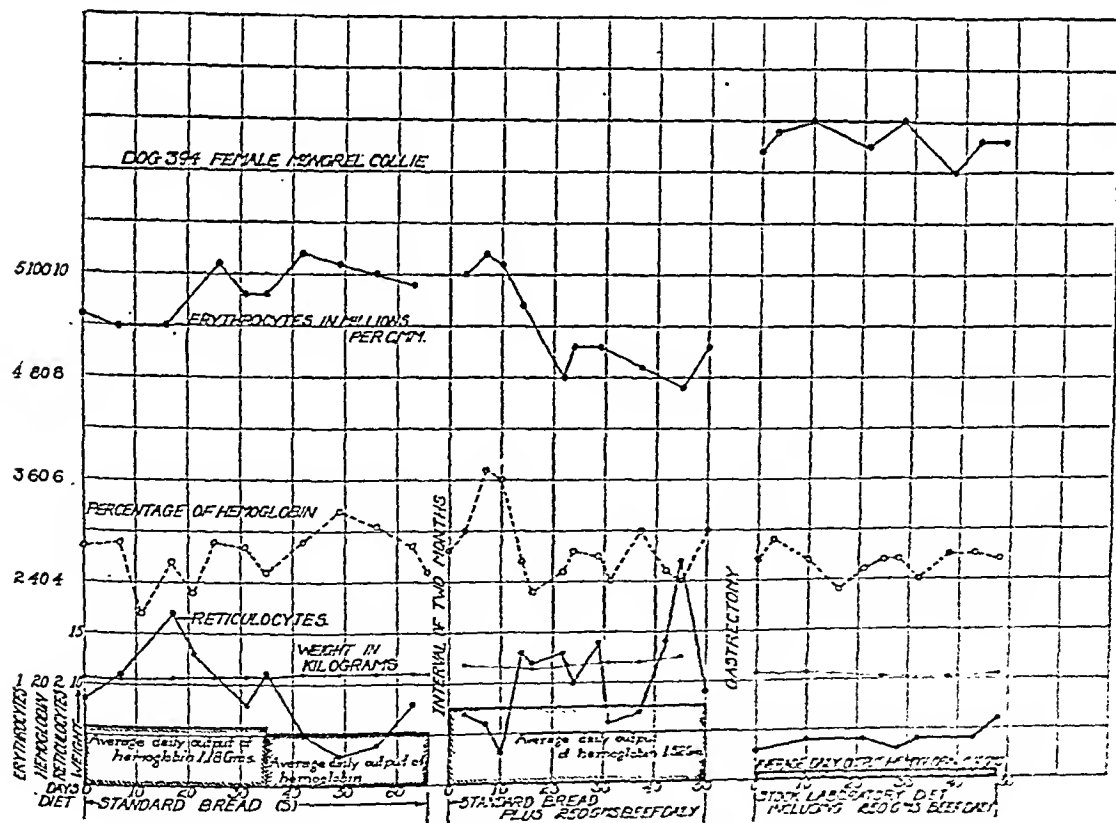


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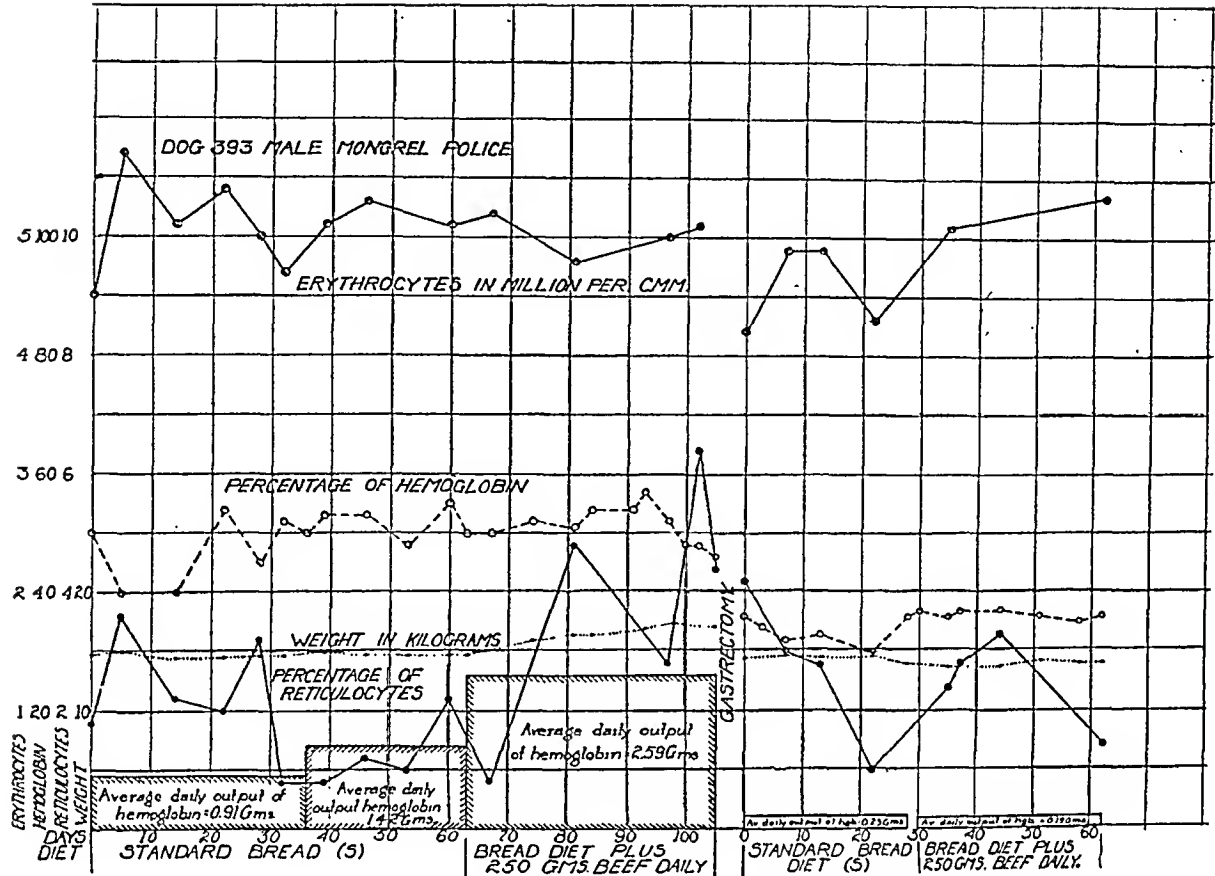


FIG. 3. SHOWS A MARKEDLY INCREASED OUTPUT OF HEMOGLOBIN ON A BEEF RATION BEFORE GASTRECTOMY, BUT A LESSENED ABILITY TO SYNTHESIZE HEMOGLOBIN FOLLOWING OPERATION.

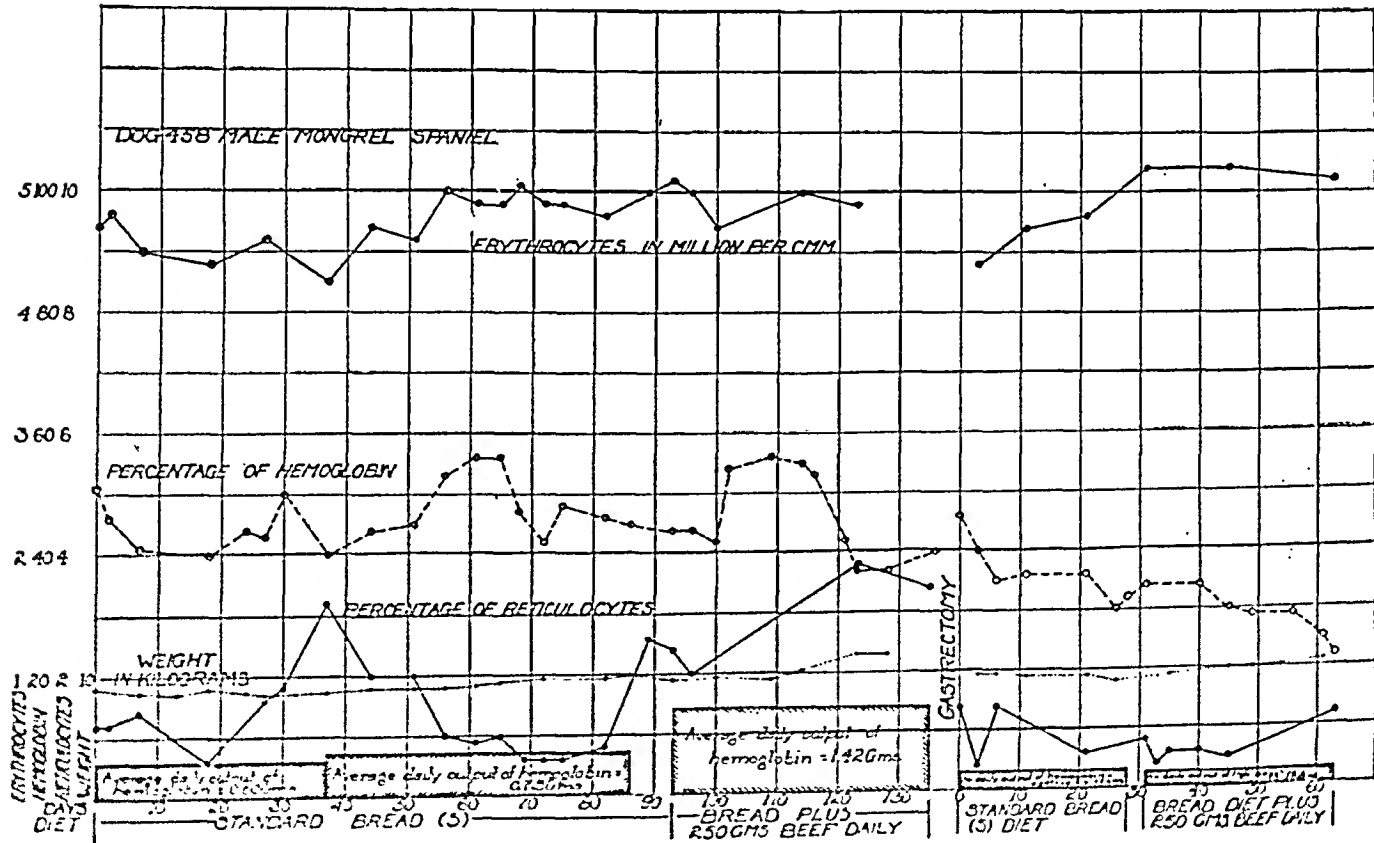


FIG. 4. SHOWS THE LESSENED ABILITY OF THE DOG TO FORM HEMOGLOBIN FOLLOWING THE SURGICAL REMOVAL OF HIS STOMACH.

It is to be emphasized that the erythrocytes remained at a relatively high level. Accordingly, the average color index was low, and the blood of the dogs was thus in a state of hypochromic anemia induced by the frequent bleedings.

TABLE I

The figures represent the quantities and volumetric aspect of the red blood cell under basal conditions of production of hemoglobin in the dog after bleeding. Each figure represents an average of several determinations on each of the four animals studied. It is to be noted that the hypochromic anemia of blood-loss became additionally microcytic after gastrectomy.

Diet	Dog number	Volume of packed red blood cells	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular concentration of hemoglobin
			cu. $\mu$	micromicrograms	per cent
Before gastrectomy					
Bread "S": 500 grams	393	29.1	58	14.5	25.0
	458	27.6	56	12.4	22.5
	473	29.5	58	12.0	20.0
	394	27.1	59	13.0	22.5
	Average	28.3	58	13.0	22.8
Bread "S": 500 grams Beef: 250 grams	393	31.9	63	15.8	25.0
	458	29.7	57	14.0	25.0
	473	26.2	51	14.9	29.0
	394	29.9	59	13.0	22.5
	Average	29.4	58	14.4	25.5
After gastrectomy					
Bread "S": 500 grams	393	27.1	57	11.3	19.0
	458	26.5	52	12.1	23.0
	473	27.6	53	12.1	23.0
	Average	27.1	54	11.8	22.0
Bread "S": 500 grams Beef: 250 grams	393	24.9	44	9.2	21.5
	458	21.9	40	7.7	19.2
	473	26.7	47	11.9	25.6
	394*	20.3	41	7.9	19.5
	Average	23.5	43	9.2	21.5

\* Stock laboratory diet including 250 grams of beef daily.

Following gastrectomy, there was little change in the mean corpuscular concentration of hemoglobin, but progressive decreases in the mean corpuscular hemoglobin occurred, as shown in Table I. Thus the anemia, which before gastrectomy was hypochromic in type, following removal of the stomach became microcytic as well.

### *The effect of the standard diet and of beef on the regeneration of hemoglobin before gastrectomy*

Since the production of hemoglobin was in a state of minimal output after bleeding the dogs over a long period of time, it was thought that a more accurate comparison of the influence of dietary factors could be made if each phase of the experiments recorded below were conducted over periods of from one to two months or longer. Accordingly, it is to be noted in Table II that the individual animals over a period of at least two months produced a daily average of 1.12, 0.68, 0.80 and 1.11 grams of hemoglobin respectively on the standard bread diet. The average daily output for the four dogs was 0.93 gram.

TABLE II

*The effect of standard bread "S" (Whipple) and of beef on the daily production of hemoglobin in dogs before and after gastrectomy. These dogs were in a state of hypochromic anemia of blood-loss, and in a condition of basal hemoglobin production.*

Diet	Dog 393		Dog 458		Dog 473		Dog 394		Average daily output of hemoglobin
	Days of observation	Daily output of hemoglobin	Days of observation	Daily output of hemoglobin	Days of observation	Daily output of hemoglobin	Days of observation	Daily output of hemoglobin	
		grams		grams		grams		grams	grams
Before gastrectomy									
Bread "S": 500 grams	63	1.12	86	0.68	70	0.80	65	1.11	0.93
Bread "S": 250 grams	42	2.59	42	1.42	34	2.25	50	1.52	1.95
Beef: 250 grams									
After gastrectomy									
Bread "S": 500 grams	23	0.23	23	-0.32	21	0.40			0.14
Bread "S": 250 grams	30	0.19	32	-0.25	37	0.21	45*	0.16	0.55
Beef: 250 grams									

\* Stock laboratory diet including 250 grams of beef daily.

Table II shows the hematopoietic reaction of the anemic dogs to a diet consisting of 250 grams of the standard bread diet, and 250 grams of lean beef. The daily output of hemoglobin on this meat diet was 2.59 grams, 1.42 grams, 2.26 grams, and 1.52 grams respectively, the average for all dogs being 1.95 grams. Thus the addition of

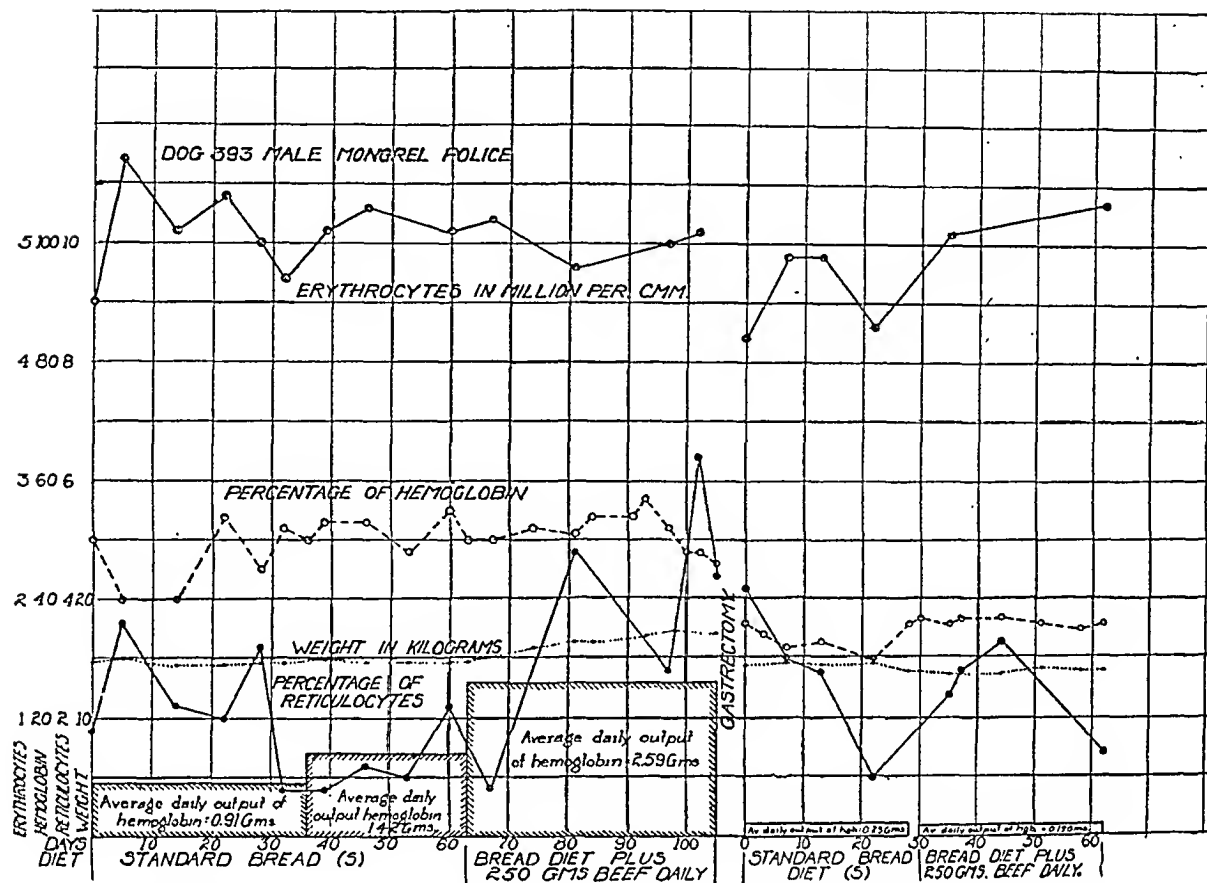


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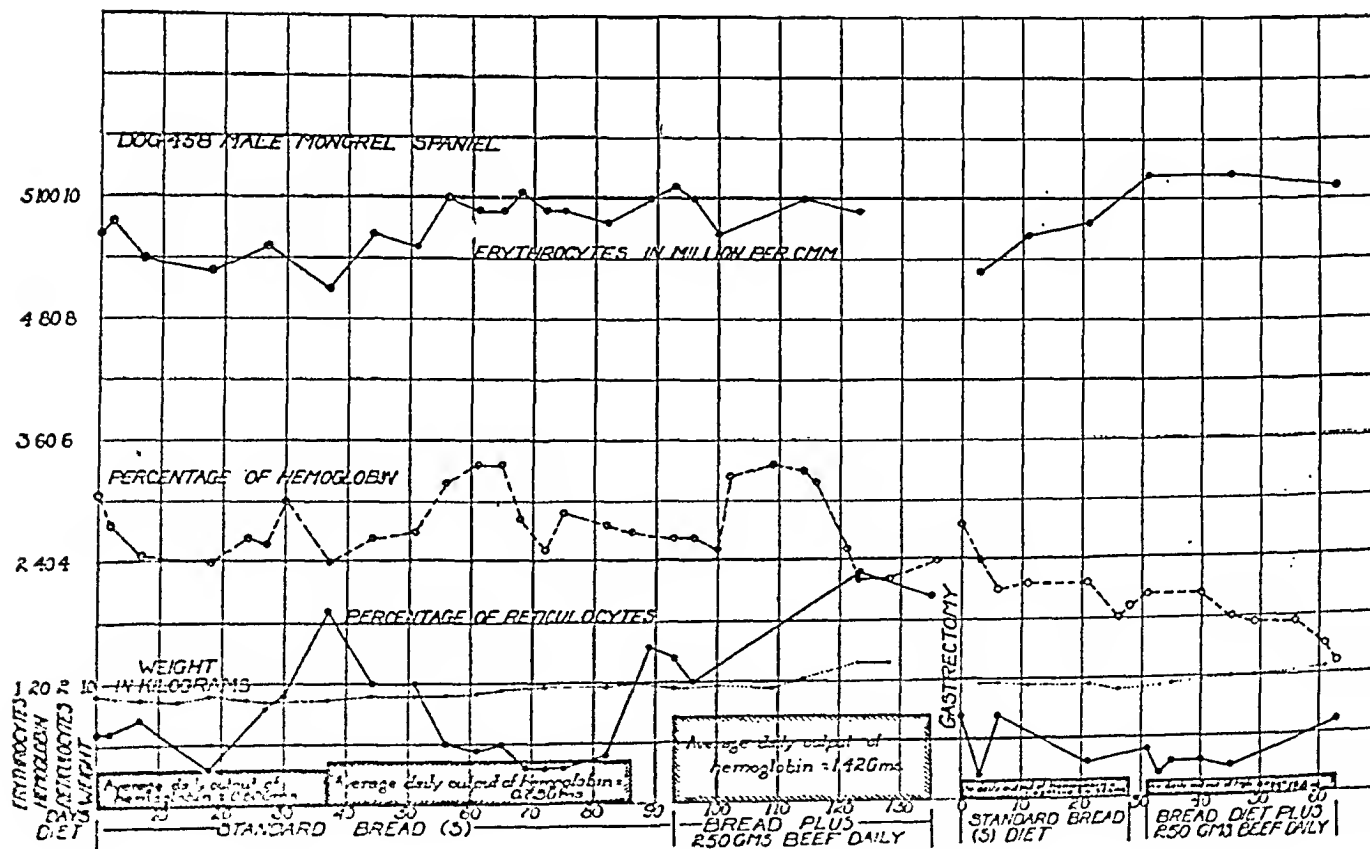


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Diet	Dog 393		Dog 453		Dog 473		Dog 394		Average daily output of hemoglobin
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Bread "S": 500 grams	63	1.12	85	0.63	70	0.80	65	1.11	0.93
Bread "S": 250 grams Beef: 250 grams	42	2.59	42	1.42	34	2.26	50	1.52	1.95
After gastrectomy									
Bread "S": 500 grams	23	0.23	23	-0.32	21	0.40			0.14
Bread "S": 250 grams Beef: 250 grams	30	0.19	32	-0.25	37	0.21	45*	0.16	0.03

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beef to the diet was followed by increases in production of hemoglobin of 130, 110, 180 and 40 per cent respectively. There was considerable individual variation both in the amount of hemoglobin produced on the basal diet and in the increases in production of hemoglobin following the addition of beef. This individual variation showed the importance of controlled preoperative observations in order to interpret accurately similar studies after gastrectomy. This reaction is comparable to that observed following the use of one of the less potent iron preparations (18), namely, ferric chloride (25 mgm. iron as metal), with a maximal weekly output of 14 grams of hemoglobin.

Our study indicates that the daily output of hemoglobin in dogs with an hypochromic anemia induced by bleeding is approximately doubled when the animal is on a meat diet, as contrasted with the standard bread ration. This is graphically illustrated in Figures 1, 2, 3 and 4. In Table I very little change may be noted in the volume of packed cells or mean corpuscular volume. However, as might be expected, slight rise of the mean corpuscular concentration of hemoglobin occurred.

#### *The effect of the standard diet and of beef on production of hemoglobin after gastrectomy*

The dogs, following the surgical removal of their stomachs, were in excellent health, and their weights tended to remain constant although at somewhat lower levels than prior to operation. Only one dog (394) refused to eat the standard bread diet, and it was found necessary to place the animal on the stock laboratory food which included 250 grams of beef fed daily.

No actual diarrhea occurred, but the stools of all animals tended to be loose or semi-solid. This is in accord with similar observations made by Ivy, Morgan and Farrell (6). The output of hemoglobin when the animals were on the bread diet fell to a low level during this phase of the experiment, as shown in Table II and Figures 1, 2, 3 and 4. The daily average was 0.14 gram, as compared to 0.93 gram before operation. The addition of meat to the diet did not bring about an increase in the production of hemoglobin. There was to be noted, on the contrary, a slight

decrease to a daily average of 0.08 gram. This decrease was slight enough, perhaps, to be within the limits of experimental error, but it indicates that dietary factors in beef favorable for formation of hemoglobin could not be utilized following gastrectomy as they were prior to operation.

#### *Spontaneous anemia*

One animal (458) developed a spontaneous anemia after gastrectomy, and showed a negative output of hemoglobin on both the bread and meat diets. In contrast to his kennel-mates, he lost weight from 10.6 to 9.0 kgm., and the level of hemoglobin fell to 3.5 grams per 100 cc. of blood. This dog died three months after operation. At autopsy no cause for death could be ascertained other than anemia. No evidence of infection or neoplasm was to be found. The duodeno-esophageal anastomosis was in good condition. The stomach tissue had been completely removed, and there was no dilatation of the duodenum. By microscopic examination, the various tissues were found to be normal, except the bone marrow. A specimen of marrow from the femur showed a marked diminution of erythropoietic elements. Much of the marrow-space was occupied by fat cells. In some of the intercellular spaces a few clumps of normoblasts and leukopoietic cells were present. Usually a marked hyperplasia of erythropoietic tissue is to be found in the marrow of dogs with anemia after bleeding (18).

#### DISCUSSION

By the experimental evidence presented, it was shown that the feeding of beef to dogs having an hypochromic anemia induced by bleeding, resulted in a marked rise in hemoglobin output. This is in agreement with the results reported by Whipple and Robscheit-Robbins (13). When, however, the stomachs of these dogs were removed and anastomoses made between the esophagus and duodenum, the regenerative power of hemoglobin was apparently greatly reduced. Before gastrectomy, the output of hemoglobin averaged 1.95 grams daily, while the dogs were on the beef diet; whereas after the operation there was an average output of only 0.08 gram. It seems probable, therefore, that the gastrectomized dogs are unable

to obtain from beef substances essential to the synthesis of hemoglobin.

Some investigators (6, 7) maintain that gastrectomy performed on the dog is not alone sufficient to induce anemia. They state that following the surgical removal of the stomach, only an occasional animal will develop a spontaneous anemia. An explanation for this may lie in the fact that dogs possess an extraordinarily large store of hematopoietic substances. It was our experience that the weekly withdrawal of from 100 to 200 cc. of blood containing from 10 to 20 grams of hemoglobin over a period of from three to eight months, was necessary before the reserves were depleted—a total of approximately 400 grams of hemoglobin. These figures varied somewhat with the individual animal. The amount of hemoglobin appears all the more significant when compared to the total hemoglobin in circulation. Such a dog as treated above, at a weight of 16 kgm., may have a circulatory blood volume of 1200 cc. If his hemoglobin level is 50 per cent, then  $12.00 \times 0.42$  equals 70.8 grams of hemoglobin in circulation. Our normal dogs on a beef diet may be expected to produce this amount of hemoglobin in five weeks.

It is difficult to understand all the chemical and mechanical factors that lead to the development of an anemia due to a deficiency of iron. However, from these experiments it would appear that, once an anemia is induced and the iron reserves depleted, this state will persist when normal gastric secretion is lacking. Clinically, this suggests that any process depleting iron stores, such as deficient diet, prolonged and profuse menses, and frequent pregnancy, in a patient with achlorhydria may lead to an hypochromic anemia, owing to the failure to get from food and to utilize properly hemoglobin-building materials.

#### CONCLUSIONS

1. The effect of feeding a standard bread ration with salmon (Whipple) on the production of hemoglobin, as compared to a diet of beef, was observed in four dogs with an hypochromic anemia induced by repeated bleeding. Subsequently gastrectomy was performed on all four animals, and again studies in the production of hemoglobin were conducted under similar dietary régimes.

2. It is shown that, prior to operation, there was an average daily output of 0.93 gram of hemoglobin on the standard bread ration, and 1.95 grams of hemoglobin after the diet was supplemented with beef.

3. Following gastrectomy, the output of hemoglobin was reduced to 0.14 gram and 0.08 gram respectively.

4. Prior to operation an hypochromic anemia was established by frequent bleeding. This became additionally microcytic following gastrectomy.

5. It is suggested that when the bodily iron reserves are depleted, a state of hypochromic anemia will persist in the absence of a normal gastric secretion, even though an iron-rich diet is ingested.

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# ADENOMA OF PANCREATIC ISLET CELLS WITH HYPOGLYCEMIA AND HYPERINSULINISM

## REPORT OF A CASE WITH STUDIES ON BLOOD SUGAR AND METABOLISM BEFORE AND AFTER OPERATIVE REMOVAL OF TUMOR

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Shortly after Banting and Best's discovery of insulin (1), Harris (10) in 1924, on the basis of cases of hypoglycemia with symptoms similar to those of insulin shock and relievable by feeding, postulated the occurrence of spontaneous "hyperinsulinism" as opposed to the "hypoinsulinism" of diabetes. The first verification of this hypothesis appeared in 1927 in a report by Wilder, Allan, Power and Robertson (26) of a case of intractable hypoglycemia. The patient, on exploration, showed carcinoma of the islet tissue of the pancreas with metastasis to the liver. Insulin was demonstrated in the hepatic metastases. Two years later Howland, Campbell, Maltby and Robinson (12) reported the first case of pancreatic islet adenoma which was diagnosed preoperatively, identified at operation and excised with favorable result.

In the past few years the literature on hyperinsulinism has grown tremendously. A review by Whipple and Frantz (25) of all the published cases of hyperinsulinism and islet tumor has appeared very recently. According to their analysis of the 157 cases reported, 75 cases of hypoglycemia were ascribed to hyperinsulinism without verification at operation or autopsy. Of the 82 remaining cases in which the pancreas was examined, normal tissue was recorded in 13 instances, hypertrophy of the islands in 4 instances and chronic inflammatory change in 3, leaving 62 instances in which a tumor of the pancreatic islands was found. Analyzing further the 62 cases of tumor, exactly 50 per cent were incidental necropsy findings without recorded hypoglycemia. The group of 31 cases of tumor associated with hypoglycemia consists of 10 discovered at autopsy and 21 at operation. Among the last 21 cases, 4 were instances of carcinoma in which death occurred shortly after operation in 2 instances.

There remain 17 patients with adenoma, the excision of which led to eventual recovery. Thus in spite of frequent reports of hypoglycemia and hyperinsulinism, adenoma of the pancreatic islets, verified at operation with favorable outcome after extirpation of the tumor is a relatively rare condition. We are reporting the history of such a case together with the results of blood sugar and metabolism studies and biological assay of the tumor for insulin.

### CASE REPORT

A Chinese man, M. T. L., aged 49 years, a mine foreman, was admitted to the Hospital on November 23, 1934, for attacks of unconsciousness and convulsions. He apparently had been well until April 1930 when, on one occasion, his consciousness became clouded after he had been awakened suddenly at night. His normal mental state returned in 3 hours after he had been given food. A similar attack occurred in September of the same year after the omission of breakfast. On that occasion he showed, in addition, haphazard movements of his limbs and profuse perspiration. Feeding again brought him out of coma. In March 1931 a strenuous journey by bicycle was followed by weakness and stiffness of the legs and collapse from which he recovered promptly after taking food. He once went to sleep without supper, and at 4 o'clock the next morning became semicomatose and then delirious, moving his arms violently, running out of his room and shouting loudly. Thereafter the attacks became more frequent, recurring approximately once every one to three months. Involuntary urination occurred during one of the attacks.

In February 1934 generalized clonic convulsions were first noticed, accompanied by coma. Subsequently similar seizures recurred almost every night, usually between 2 and 4 a.m. According to the description given by his wife, the onset of the seizures was marked by a cessation of the snoring which occurred regularly during sleep and the opening of his eyes accompanied by staring and grimacing. Twitching movements first involved the lower limbs and then the upper. In the beginning the movements were slight and then more violent. Occasionally the tongue was bitten. Each series of

vulsions usually lasted a few minutes and would be followed by an interval of relative quietness during which the patient could be induced to swallow some carbohydrate foods which his wife had learned to feed him in order to abolish the attacks.

During the intervals between the attacks the patient apparently was normal except for an impaired memory of recent events. He grew very fat during the two years prior to admission, his appetite being enormously increased. There was no loss of libido or potency. He had abandoned his work in 1932.

In the past history there was a record of smallpox at the age of 12, dysentery at 37, urethral discharge at 38, and an acute febrile illness of unknown nature at 42.

*Physical examination.* Between attacks the patient appeared perfectly comfortable. Temperature 37.4° C., pulse 80, blood pressure 104/70, height 172 cm., weight 96.7 kgm. The patient was well developed and markedly obese, with fat distributed generally without special predilection for given regions. Distribution of hair was normal except for a tendency toward the feminine type in the pubic region. The ocular fundi showed a slight degree of perivasculitis of the retinal vessels; visual fields normal. Thyroid gland was not enlarged. Lungs were normal. Heart was not enlarged, and the radial arteries were not thickened. Masses or individual organs could not be palpated within the abdomen. External genitalia were normal; prostate gland not enlarged. Neurological examination revealed nothing of significance. Dr. H. I. Chu, who saw the patient first in the Outpatient Clinic, suggested the diagnosis of hypoglycemic syndrome.

*Laboratory data.* Urinalysis gave normal findings and stools proved negative for ova or parasites. The red blood count was 5.9 million; hemoglobin, 15.3 grams; and white blood cells, 12,100 with 67 per cent neutrophils. The blood sugar during the postabsorptive period as determined on many occasions was between 40 and 50 mgm. per cent. Icterus index was 6. A bromsulphalein test revealed no retention of the dye after 30 minutes. Roentgenologic examination of the skull showed a normal sella turcica. The basal metabolic rate was 1,977 calories per diem or +3.0 per cent. Blood Wassermann reaction was negative.

The ordinary three-meal diet was not adequate to prevent convulsive seizures which usually came between 4 and 5 a.m. The patient therefore was given a diet containing 3,000 calories with 473 grams carbohydrates divided into 5 equal portions served at 8 a.m., 12 noon, 5 p.m., 10 p.m. and 3 a.m. This regimen kept him free from symptoms for a few days, but after that mild attacks re-appeared before the 3 a.m. and 8 a.m. feedings, and the diet had to be increased to 3,500 calories with 600 grams carbohydrates.

During the interval between admission and operation various studies on blood sugar and metabolism were made, which are described later.

*Operation.* On January 2, 1935, with the patient under ether anesthesia a laparotomy was performed (H. H. L.).

The abdomen was opened through a left rectus incision extending from the xiphoid process to a point about 3 cm. below the umbilicus. The subcutaneous and omental fat was large in quantity and a portion of the greater omentum was excised in order to facilitate exposure. The usual exploration revealed the fact that the liver was small with smooth surfaces and a thin, almost knife-like edge. The gallbladder, stomach, duodenum, small intestine, appendix and colon all appeared normal. The spleen was small. It was difficult to palpate the kidneys on account of excessive perirenal fat.

A duodenal tube was passed to deflate the stomach, and the pancreas was approached through the gastrocolic omentum. Along the upper margin of the right half of the body of the pancreas at approximately the point of junction of the head and body lay a tumor 2.5 cm. in diameter which was somewhat firmer in consistency than the rest of the gland. It was dark red in color and was elevated slightly above the surface of the gland. It appeared to be contained within a thin capsule and was enucleated easily from its bed in the pancreas. Numerous large veins ran between the capsule and the substance of the gland and hemostasis presented some difficulty, ligatures and the coagulating current both being employed. An iodoform gauze drain was packed lightly into the denuded area, and two cigarette drains were placed down to the level of the pancreas. The gastrocolic omentum was closed by interrupted sutures of fine silk. The abdominal wall was closed in layers around the drains, the closure being reinforced by three stay sutures of silver wire.

*Postoperative course.* For some hours prior to and during the operation, as well as for some hours subsequently, the patient was given a 5 per cent solution of glucose in normal saline by means of a continuous intravenous drip (40 drops per minute). The blood sugar at 9 a.m. (before operation) was 59, and at 1 p.m. (shortly after operation) it was 158; later in the day it rose to 264 mgm. per cent and sugar appeared in the urine. Temperature 39.3° C.; pulse 130. Glucose by intravenous drip was discontinued and 400 cc. of citrated blood were given.

On the next day the temperature went higher (40.4° C.), and the respirations increased to 34 per minute. Aside from slight cough and a few râles heard at the bases of both lungs, there was nothing to indicate a pulmonary complication. A moderate bloody discharge drained from the operative wound. One cigarette drain was removed. The white blood cell count was 8,850. The blood sugar was maintained between 175 and 245 mgm. per cent, and the urinary sugar totaled 4 grams in 24 hours. Insulin was started, and given in doses of ten units three times a day.

On the third day the fever began to subside, the general condition improving. The blood sugar was 238 to 300 mgm. per cent; the urine sugar 17 grams. The iodoform gauze drain was removed.

On the fourth day the patient was well enough to take a high calorie diet. The blood sugar was 276 mgm.

per cent and the urinary sugar totaled 22 grams. On the fifth day the temperature became normal. The last drain was removed and some serosanguineous discharge came from the wound. The hyperglycemia and glycosuria tended to diminish. Insulin was discontinued on the 7th day, and the blood sugar became normal and the urine sugar-free on the 9th day, before a calculated diabetic diet was started. From then on convalescence was uncomplicated, the wound closing gradually and the discharge becoming less in amount. The patient was up and about three weeks after the operation and did not experience any of the symptoms which had been present previously. A number of the preoperative observations on blood sugar and metabolism were repeated, and the results are described later. Although the patient showed evidence of a slight diabetic tendency as indicated by a sugar tolerance test, he tolerated a full diet with a normal fasting blood sugar and without glycosuria. He was discharged on February 22, 1935, in good condition except for a small sinus at the upper part of the wound which still drained thin fluid resembling pancreatic juice. His weight was 81.8 kgm., a loss of 14.9 kgm. compared with that on admission.

Examination on May 20, 1935, five months after operation, showed that the patient was well, and was without recurrence of the preoperative symptoms. The sinus had closed shortly after discharge. The fasting blood sugar was 111 mgm. per cent. A 24-hour specimen of urine contained no sugar. The weight was 80.8 kgm.



FIG. 1. GROSS APPEARANCE OF THE TUMOR REMOVED FROM THE PANCREAS.

#### STUDIES OF THE TUMOR

**Anatomical observations.** The tumor as shown in Figure 1 appeared nodular, dark red in color, and firm in consistency, measuring  $2.5 \times 2.0 \times 1.5$  cm. in diameter. It weighed 4.41 grams. The anterior surface was covered by a

thin fibrous capsule, while its posterior surface, i.e., that portion of the tumor in contact with the gland, was studded with small areas of pancreatic tissue. The cut surface was grayish, cellular in appearance and showed many congested blood vessels. Microscopically the tumor consisted of a diffuse solid growth of cells which were uniform in size, well differentiated and morphologically identical with those of Langerhans' islands (Figure 2). In some areas the cells were arranged in cords or trabeculae much like the structure of normal islands. Mitotic figures were not seen. The anterior surface was entirely encapsulated, but the posterior surface was cut across irregularly, and in certain areas, immediately beneath the capsule groups of pancreatic acini were present.

**Biologic assay for insulin.** The method of extraction used was that of Best, Jephcott and Scott (2). A portion of the tumor weighing 1.92 grams was extracted, and the volume of extract made up to 20 cc. The assay was done on two rabbits of comparable weight which had been deprived of food for 24 hours prior to injection. As seen from Table I, the extent and duration of hypo-

TABLE I

Results of assay of extract of the tumor for insulin on rabbits

	Blood sugar		
	Control	2 hours	4 hours
	mgm. per cent	mgm. per cent	mgm. per cent
Rabbit 1			
January 4, weight 1570 grams, 0.5 cc. of insulin (0.5 unit).....	141.4	75.7	117.1
January 6, weight 1510 grams, 0.5 cc. of extract (0.05 gram).....	124.2	72.2	101.9
Rabbit 2			
January 4, weight 1820 grams, 0.5 cc. of extract (0.05 gram).....	138.5	83.6	111.1
January 6, weight 1724 grams, 0.5 cc. of insulin (0.5 unit).....	117.1	78.2	118.8

glycemia in both rabbits after the injection of tumor extract happened to approximate those after 0.5 unit of insulin. Therefore the tumor tissue was considered to contain approximately 10 units of insulin per gram. According to Best, Jephcott and Scott, beef pancreas yields on the

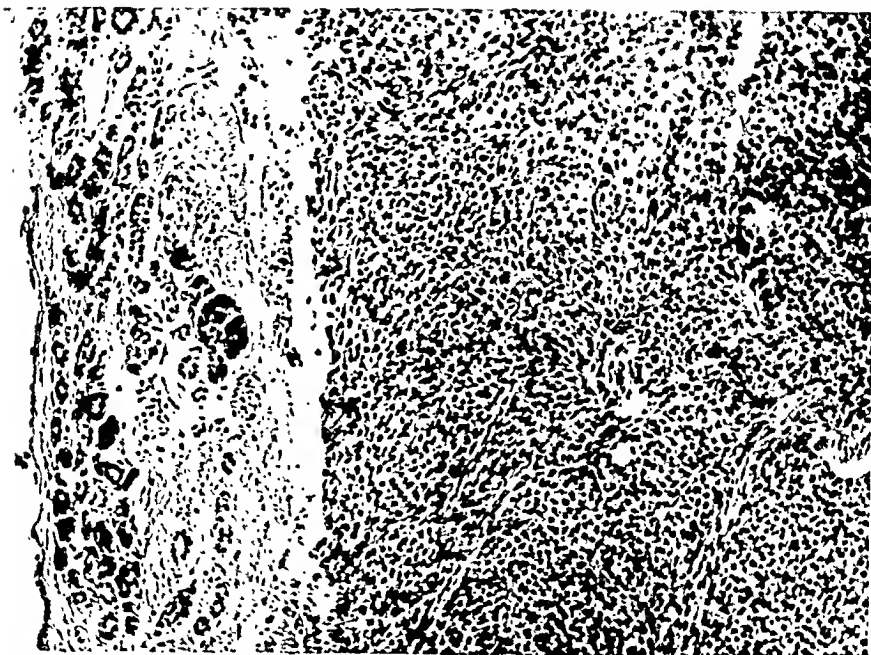


FIG. 2 A. PHOTOMICROGRAPH OF A SECTION NEAR THE PERIPHERY OF THE TUMOR SHOWING ACINAR TISSUE IN THE CAPSULE (LEFT) AND TUMOR TISSUE WITH TRABECULA FORMATION (RIGHT).

Magnification 111 times.

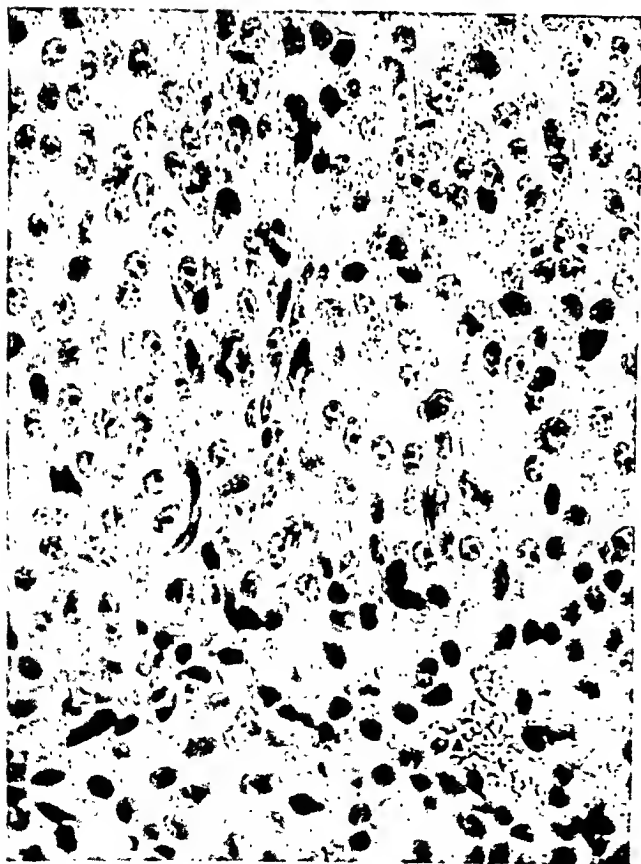


FIG. 2 B. PHOTOMICROGRAPH OF A SECTION SHOWING CYTOLOGY OF THE TUMOR IN GREATER DETAIL.

Magnification 445 times.

average 3,000 units of insulin per kgm. or 3 units per gram as determined by their method. The high insulin content of the tumor justifies the diagnosis of hyperinsulinism in this patient.

In the literature, there are recorded three successful attempts to extract insulin or an insulin-like substance from a tumor of the pancreas removed at operation (5, 8, 26). It appears that the tumor in our case had a higher insulin content than that of the tumors previously reported.

#### OBSERVATIONS ON THE BLOOD SUGAR AND RESPIRATORY METABOLISM

*Behavior of blood sugar in relation to meals.* While the patient was being given a diet of 3,000 calories with 473 grams of carbohydrate distributed evenly among five meals, the capillary blood sugar was determined hourly throughout 24 hours by the Folin-Wu method (7) adapted to 0.1 cc. samples. The results are plotted in Figure 3, from which one notes that the blood sugar rose to a maximum within 1 to 3 hours after each meal. The maxima, which varied from 65 to 115 mgm. per cent, were below normal after meals, although the values were low to start with. The more significant abnormality lies in the fail-

ure of the blood sugar to maintain a normal level during the postabsorptive period. Within 4 to 5 hours after each meal when absorption could

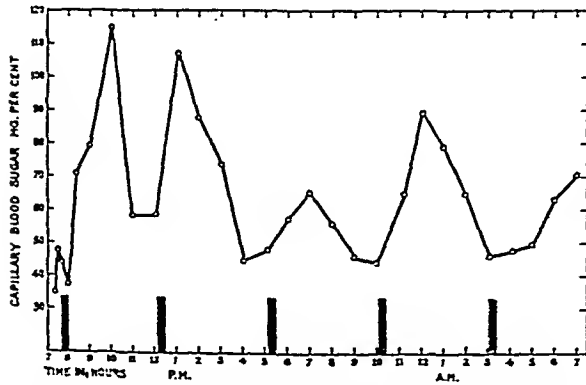


FIG. 3. HOURLY BLOOD SUGAR THROUGHOUT 24 HOURS IN RELATION TO MEALS WHICH ARE INDICATED BY VERTICAL BARS.

hardly be complete it promptly fell to 44 to 58 mgm. per cent. If, therefore, meals had been delayed, it is reasonable to suppose that the level would have continued to fall until symptoms occurred. Therefore, the condition can be characterized as one of pronounced postabsorptive hypoglycemia in which frequent feedings were necessary to prevent the development of symptoms.

*Correlation between the level of blood sugar*

*and the clinical picture.* On one morning with breakfast omitted, the patient was observed carefully, and the capillary blood sugar, blood pressure and pulse rate were determined at approximately 10-minute intervals. As seen from Figure 4, the observations were commenced at 8:00 a.m. when the patient was quiet and mentally clear with the blood sugar fluctuating at 45 to 50 mgm. per cent. He remained in this condition for about 25 to 30 minutes, after which without appreciable change in the level of the blood sugar he became slightly drowsy, but easily arousable. The drowsiness gradually deepened so that in another 30 minutes when awakened he again fell asleep and began snoring at once. The blood sugar level remained practically unchanged. The period of profound drowsiness lasted 25 minutes before it passed into a stage of excitement. Then the patient became restless, opened his eyes in a staring manner and yawned repeatedly. The fists were clenched tightly, and the legs were moved about purposelessly. At times, fine twitchings of the muscles of the hands and face were noticed. The patient still seemed to recognize loud calls or commands, but was unable to answer or act upon them. The blood sugar level tended to fall somewhat, slightly below 45 mgm. per cent. This period lasted 10 minutes before active generalized clonic convul-

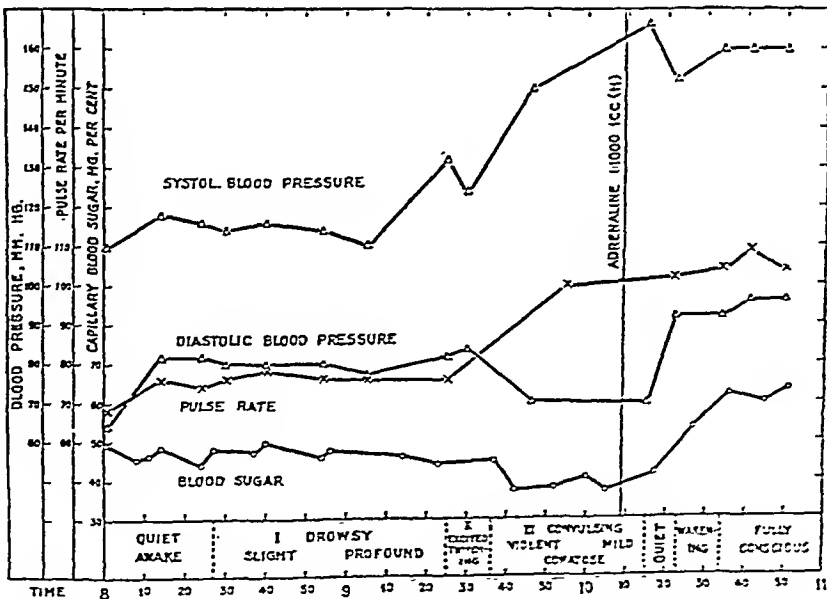


FIG. 4. CORRELATION BETWEEN LEVEL OF BLOOD SUGAR AND MENTAL STATE. BLOOD PRESSURE AND PULSE RATE ALSO INDICATED.



sions began. The convulsions increased gradually in violence, the patient's whole body being thrown about the bed with such force that several men were required to hold him down. Breathing was stertorous, with frothing at the mouth. Violent seizures continued for 15 minutes before they gave place to milder convulsive movements of the limbs. With the onset of convulsions the blood sugar dropped to about 37 to 40 mgm. per cent. The blood pressure began to rise during the excitement and reached 150/70 during the convulsions. The pulse rate also was increased. One cubic centimeter of 1:1,000 solution of adrenalin given subcutaneously was followed in 5 minutes by a subsidence of the convulsions and in 15 minutes, by consciousness.

It seems that the hypoglycemic syndrome in this patient can be divided conveniently into three stages. The first stage was characterized by drowsiness and a gradually deepening depression. The second stage was ushered in by excitement which progressed into the convulsive seizures of the third stage. During the first stage which lasted for an hour there was no significant decrease of blood sugar compared with that of the period of relative well-being. Even when the second stage was reached, the lowering of blood sugar was slight, if any. It was not until after the convulsions had started that definite further lowering of the blood sugar occurred. From these observations it may be inferred that hypoglycemia has to be maintained for a certain length of time, which in this patient amounted to over two hours, before the central nervous system suffers sufficiently to manifest itself by convulsions. It seems that besides the level of the hypoglycemia, the duration is an important determining factor in the symptomatic manifestations. Unfortunately, our observations did not extend over a sufficiently long period prior to the symptoms to enable us to estimate accurately the duration of the critical level of blood sugar necessary to initiate an attack.

*The effect of adrenalin.* A detailed study of the blood sugar level and its relation to symptomatic manifestations following the administration of adrenalin is given in Figure 5. The efficacy of adrenalin in relieving the hypoglycemic syndrome, a well known phenomenon, was demonstrated clearly in this case. With the patient

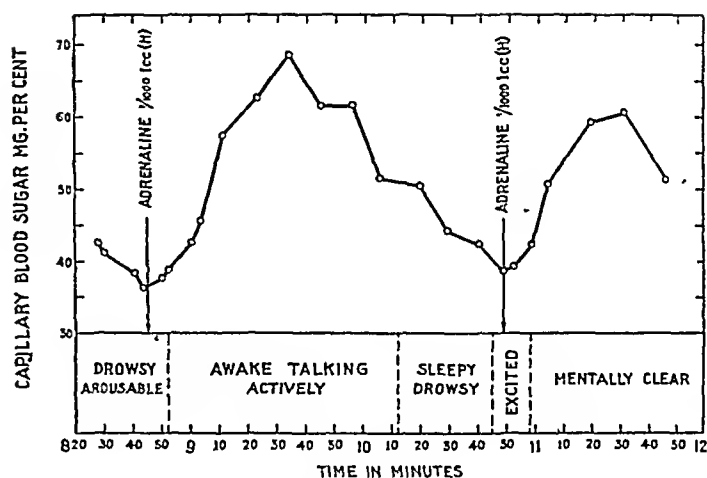


FIG. 5. EFFICACY OF ADRENALIN IN RAISING BLOOD SUGAR AND RELIEVING SYMPTOMS.

mentally drowsy and the blood sugar at the low level of 36 mgm. per cent, 1 cc. of adrenalin injected hypodermically was followed within 10 minutes by a return of consciousness associated with a rise of the blood sugar to 42 mgm. per cent. A maximum level of 68.6 mgm. per cent was reached about 45 minutes after the injection. From then on it commenced to fall, and 85 minutes after the injection it reached a level of approximately 50 mgm. per cent at which time the patient again began to feel drowsy. Two hours after the injection the blood sugar was back to the original low level, and the patient once more was in a semicomatose condition. A second injection of adrenalin at this point brought about a similar reaction, although the maximum level of blood sugar attained was somewhat lower.

It may be noted here that there apparently was a difference in the blood sugar level referable to a given mental state depending on whether the blood sugar was increasing or decreasing. During the ascent of the curve following the injection of adrenalin, consciousness was almost regained at 42 mgm. per cent, while during its descent drowsiness supervened at 50 mgm. per cent. The difference was probably significant, although no attempt was made to determine the true glucose content of blood which might not have shown such discrepancies.

The action of adrenalin was studied further by the simultaneous determination of the venous blood sugar and serum inorganic phosphorus (6) following the administration of this drug both preoperatively and after convalescence from the operation. As shown in Figure 6, the adrenalin

hyperglycemia before operation, as in previous observations, reached its peak 40 minutes after injection, and the blood sugar returned to the in-

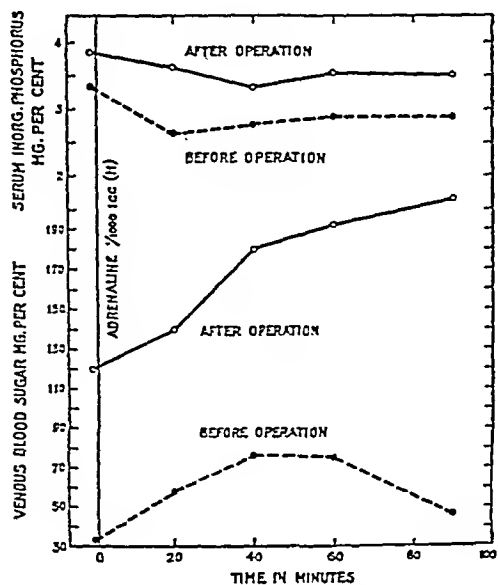


FIG. 6. COMPARISON OF THE EFFECT OF ADRENALIN ON BLOOD SUGAR AND SERUM INORGANIC PHOSPHORUS BEFORE AND AFTER OPERATIVE REMOVAL OF THE TUMOR OF ISLANDS OF LANGERHANS.

ital level in 90 minutes. However, after operation, at which the source of excess insulin was removed, adrenalin caused a much more extensive and prolonged rise in the blood sugar; at 90 minutes after injection when the study was terminated, a maximum value of 198 mgm. per cent was obtained. The serum inorganic phosphorus curve before operation was on a lower level and showed a more definite decline following the administration of adrenalin than that obtained by the same procedure after excision of the tumor.

These observations may be summarized by saying that although adrenalin was very promptly effective in combating the hypoglycemic syndrome, thus acting as a true antagonist to insulin, its action was considerably curtailed and shortened in the presence of excess insulin. The fact that adrenalin was repeatedly effective indicated that a considerable store of glycogen in the liver or other tissues was available for conversion into glucose.

*Effect of pituitrin, pitressin, and ephedrine.* One cubic centimeter of pituitrin (Parke, Davis

and Co.) was given hypodermically on one occasion when the blood sugar was only 38 mgm. per cent and the patient was emerging from drowsiness into excitement; 15 minutes later the blood sugar rose to 42 mgm. per cent, and the patient was somewhat clearer for a short while (10 minutes) before returning to the pre-injection state. One and a half cubic centimeters of pitressin (Parke, Davis and Co.) was then given subcutaneously, and its action was observed to be similarly slight and doubtful. On another occasion ephedrine was used (100 mgm. hypodermically) without the slightest effect on the course of the patient's symptoms or the level of the blood sugar.

*Effect of levulose.* One morning when the patient was slightly drowsy and the blood sugar was 48 mgm. per cent, he was given, instead of his usual breakfast, 50 grams of levulose dissolved in 250 cc. of water. The drowsiness persisted and gradually deepened and 70 minutes after the ingestion of levulose restlessness set in and progressed to mild convulsive attacks. The blood sugar, however, rose to 55 mgm. per cent in 30 minutes and fell to 44 mgm. per cent in 70 minutes at which time mild convulsions supervened. This ineffectiveness of levulose in combating the hypoglycemic syndrome is in conformity with the results of Cori (3) who showed that levulose cannot be utilized by the tissues until it first has been converted into glycogen by the liver. The slight rise of blood sugar probably represented levulose which was ineffective in counteracting the symptoms.

*Comparison of the effect of glucose per rectum and by mouth.* Although glucose often has been administered per rectum, doubt still exists as to whether it is absorbed through this route. Our patient afforded an excellent indicator with which to test the efficacy of the absorption of glucose by rectum inasmuch as very slight changes in the level of the blood sugar were manifested by marked changes in his mental state. As indicated in Figure 7, 600 cc. of 10 per cent solution of glucose given per rectum in three installments within a period of one hour did not prevent the gradual lowering of the blood sugar or the progression of drowsiness to the subsequent state of excitement and convulsions. This is a sharp contrast to the efficacy of glucose administered by mouth, by which route even much smaller amounts

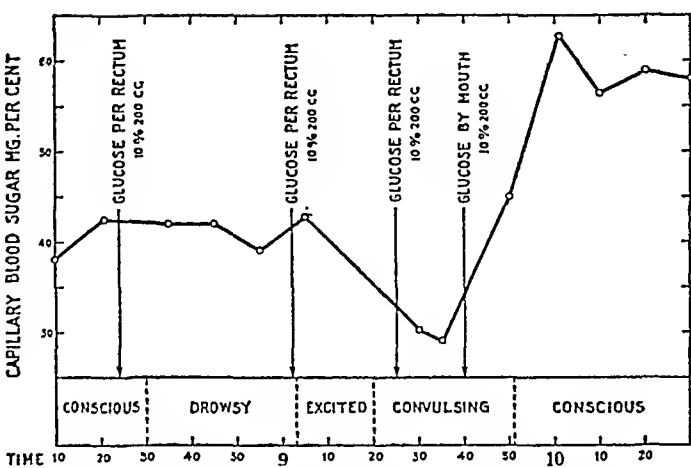


FIG. 7. INEFFECTIVENESS OF GLUCOSE PER RECTUM IN COMBATING THE HYPOLYCEMIC SYNDROME IN CONTRAST TO THE EFFICACY OF GLUCOSE BY MOUTH.

of glucose brought about immediate amelioration of symptoms and a rise of the blood sugar. This observation indicates that glucose in this concentration per rectum is not absorbed or is absorbed so slowly that physiologic effects are not demonstrable. This conclusion is in agreement with that of Scott and Zweighaft (21).

*Effect of glucose on blood sugar, metabolic rate and respiratory quotient before and after operation.* The study consisted of half-hourly analyses of the capillary blood sugar, hourly determinations of the metabolic rate and estimations of urinary nitrogen, both before and for 4 hours after the ingestion of 170 grams of glucose. The expiratory gas was collected in a Tissot gasometer for a period of 10 to 15 minutes each time and analyzed for  $O_2$  and  $CO_2$  in a Haldane apparatus. Urinary nitrogen was determined by the Kjeldahl method. The data on blood sugar, respiratory quotient, caloric output, and calculated composition of metabolic mixture during the experimental periods are presented in Figure 8 and Tables II and III.

In Figure 8, the blood sugar curve before operation is seen to deviate from normal in two respects. First, the peak (156 mgm. per cent) was not reached until two hours after the ingestion of glucose. Second, after a normal level had been reached at the end of three hours the curve continued to fall to the level of 51 mgm. per cent at the end of the fourth hour, a low level comparable to that which existed before glucose was administered. It seems that the excess insulin present in the blood stream prevented a more prompt

attainment of the maximal level. As soon as this was reached, more insulin was called forth. The excess insulin together with the diminishing amount of glucose entering the blood stream from

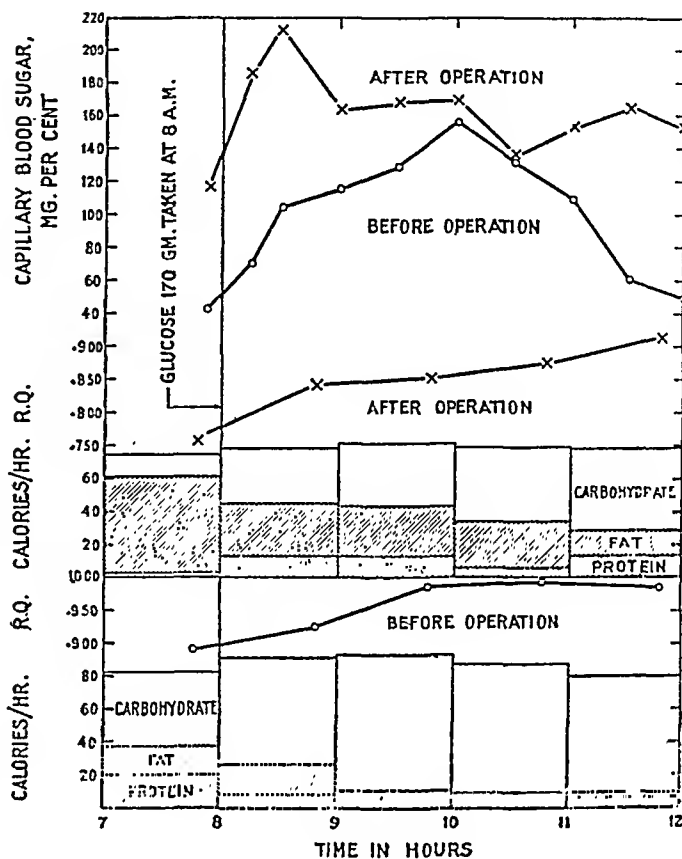


FIG. 8. THE EFFECT OF GLUCOSE INGESTION ON BLOOD SUGAR, RESPIRATORY QUOTIENT, METABOLIC RATE, AND COMPOSITION OF THE METABOLIC MIXTURE BEFORE AND AFTER OPERATION.

TABLE II

*Effect on respiratory quotient and metabolic rate of 170 grams glucose ingested at 8:00 a.m.*

Time	Total respiratory quotient		Nonprotein respiratory quotient		Calories per hour		Calories per 24 hours		Meta-bolic rate		Increase over basal rate	
	A*	P†	A*	P†	A*	P†	A*	P†	A*	P†	A*	P†
a.m.									per cent	per cent	per cent	per cent
7-8	0.883	0.765	0.910	0.763	82.4	74.7	1977	1793	3.0	0.0	0	0.0
8-9	0.925	0.848	0.937	0.835	91.5	77.7	2197	1866	14.4	4.0	11.0	4.0
9-11	0.957	0.856	1.010	0.866	92.1	80.8	2210	1941	15.1	8.2	11.8	8.2
10-11	0.992	0.878	1.014	0.884	86.6	78.6	2070	1885	8.2	5.2	5.1	5.2
11-12	0.972	0.914	0.937	0.931	80.1	78.8	1924	1892	0.1	5.5	-2.8	5.5

\* A, before operation, height 172 cm., weight 95.0 kgm., surface area 2.08 square meters, Aub-DuBois standard 80.0 calories per hour.

† P, after operation, height 172 cm., weight 82 kgm., surface area 1.94 square meters, Aub-DuBois standard 74.7 calories per hour.

TABLE III

*Combustion of protein (from urinary nitrogen), carbohydrate and fat (from nonprotein respiratory quotient) before and after ingestion of 170 grams glucose at 8 a.m.\**

Time	Protein				Carbohydrate				Fat			
	A†		P‡		A†		P‡		A†		P‡	
a.m.	grams	calories	grams	calories	grams	calories	grams	calories	grams	calories	grams	calories
7-8	4.48	19.0	0.46	2.0	11.0	46.0	3.4	14.1	1.84	17.4	6.19	58.6
8-9	1.76	7.4	2.97	12.6	16.0	65.9	7.9	33.0	1.94	18.2	3.39	32.1
9-10	2.30	9.7	2.79	12.8	19.7	82.4	8.8	37.0	0.0	0.0	3.27	31.0
10-11	1.98	8.4	1.34	5.3	18.7	78.2	11.6	44.4	0.0	0.0	3.06	28.9
11-12	1.37	5.8	3.12	13.2	17.0	71.0	12.0	50.2	0.35	3.3	1.63	15.4
Total for last 4 hours	7.41	31.3	10.22	43.9	71.4	297.5	40.3	164.6	2.29	21.5	11.35	107.4

\* Calculation according to Lusk (16); protein equivalent to 4.24 calories; carbohydrate 4.18 calories; and fat 9.46 calories per gram.

† A, before operation, 42.6 per cent of the glucose ingested was burned during 4 hours; protein contributed 8.9 per cent, carbohydrate 85.0 per cent, and fat 6.1 per cent of the total energy metabolism during 4 hours subsequent to glucose administration.

‡ P, after operation, 23.7 per cent of the glucose ingested was burned during 4 hours; protein contributed 13.8 per cent, carbohydrate 52.1 per cent, and fat 34.1 per cent of the total energy metabolism during 4 hours subsequent to glucose administration.

the gastro-intestinal tract drove the blood sugar down to the low level. The curve obtained after operation was markedly different in that the peak of 214 mgm. per cent was reached in half an hour and a level between 133 and 169 mgm. per cent was maintained during the subsequent 3 hours. The high maximum and sustained hyperglycemia indicate an impaired mechanism for the removal of glucose suggestive of the prediabetic state, although the fasting blood sugar was normal and urinary sugar was absent.

The metabolic rate increased after the administration of glucose, the extent of the increase (specific dynamic action) being greater before (12 per cent) than after operation (8 per cent). During the period of hyperinsulinism the respiratory quotient before glucose (0.883) was higher than normal and went up nearly to unity after glucose administration, while after operation the quotient before glucose (0.765) was lower than normal and rose only to 0.914 during the fourth hour after the ingestion of this substance. The marked lowering of the respiratory quotient after operation indicates the profound changes in the composition of the metabolic mixture brought about by the removal of the pancreatic adenoma.

Prior to operation and during the 4 hours following the ingestion of 170 grams of glucose, 71.4 grams carbohydrate, 7.4 grams protein and 2.3 grams fat were burned; while after operation,

40.3 grams carbohydrate, 10.2 grams protein and 11.4 grams fat were metabolized under comparable conditions (Table III, Figure 8). In other words, prior to operation, when there was an excess of insulin the metabolism of carbohydrates played a more dominating rôle and proceeded at a faster rate in contrast to the situation after operation in which carbohydrate was used more slowly and protein and fat were drawn upon to a greater extent.

Linder, Hiller and Van Slyke (15) fed 90 to 100 grams of glucose to normal fasting men, and determined their respiratory quotients and total metabolism. In four hours only 20 to 30 per cent of the glucose had been burned, although apparently all had been absorbed. In our patient glucose combustion prior to operation was 42.6 per cent, exceeding normal limits; while after operation it amounted to 23.7 per cent, which was fairly normal.

The sparing of fat and protein as a result of the predominant utilization of carbohydrate by virtue of the excess insulin, together with over-eating, probably accounts for the marked obesity this patient exhibited prior to surgical treatment.

#### COMMENT

The hypoglycemic syndrome is a symptom complex associated with abnormally low blood sugar. The level of blood sugar at which symptoms oc-

cur varies from case to case. Of the large series of cases of hyperinsulinism compiled by Whipple and Frantz (25), the minimal blood sugar ranged from 4 to 58, with the majority falling between 30 and 45 mgm. per cent. The wide range is accounted for partly by the different methods used in the determinations and partly by individual susceptibility to the effects of hypoglycemia. This susceptibility apparently varies not only with age but with other factors as well, so that different responses may be obtained from the same individual on different occasions. Moreover, the ascending threshold for clear consciousness is lower than the descending threshold. There was a difference of over 15 mgm. per cent in our patient.

The symptoms associated with hypoglycemia are protean. If a careful history is taken, the attacks are found almost always to be associated with omission or delay of meals. Overpowering hunger and weakness frequently initiate the trouble, and the patients or their relatives discover sooner or later that food aborts or relieves attacks. Often larger and more frequent meals are taken to prevent symptoms. Overeating together with the protein- and fat-sparing action of insulin results in obesity.

The symptoms of hypoglycemia, though variable, are chiefly vasomotor, such as sweating, flushes, and an increase in the pulse rate and blood pressure; and psychic disturbances, such as drowsiness, excitement, delirium, maniacal seizures, convulsions and coma. Weakness and fatigue are prominent. Ryneerson and Moersch (20) tabulated the symptoms. The picture during an attack may vary in individual cases.

From the extensive literature on carbohydrate metabolism reviewed by Cori (4) and Shaffer and Ronzoni (22), it has become generally accepted that the blood sugar is maintained normally at a concentration of approximately 100 mgm. per cent mainly through the interplay between insulin and epinephrine. A rise in blood sugar elicits a secretion of insulin which accelerates conversion of glucose into glycogen and carbohydrate oxidation in the liver, muscles and presumably all tissues, while a fall in blood sugar is followed by a discharge of epinephrine which increases the rate of hydrolysis of liver glycogen to glucose and

conversion of muscle glycogen lactate. The latter is reconverted into glycogen by the liver thereby contributing indirectly to the sugar of the blood. Macleod (18) postulates a sugar regulating center in the pons. Its stimulation by hyperglycemia causes the secretion of insulin by way of the vagus; while hypoglycemia results in sympathetic stimulation and the outpouring of epinephrine.

Wauchope (24) has summarized the causes of hypoglycemia under four main headings: First, hyperinsulinism which may be functional, or on the basis of adenoma, carcinoma or hyperplasia of pancreatic islets; or therapeutic, as a result of an overdosage of insulin. Second, lack of opposing hormones, as in Addison's disease, myxedema, and pituitary cachexia. Third, deficient glycogen store due to liver disease (13, 14), severe exercise, excessive drain (renal diabetes or lactation), or starvation. Fourth, disturbances of the regulating center in the pons (vagotonia).

Before a diagnosis of hyperinsulinism is made, all the other conditions enumerated above should be considered and excluded. This was done in our case without much difficulty. The finding of an adenoma of the pancreas composed of cells of the islands of Langerhans, the abatement of symptoms after excision of the tumor and finally the presence of large amounts of insulin in the biological assay of the tumor tissue established beyond doubt that the anatomical basis of the hypoglycemia in our patient was an islet cell adenoma.

As regards treatment, frequent meals rich in carbohydrate usually suffice to avert the symptoms when these are slight and infrequent. Harris (11), however, considers that a diet relatively poor in carbohydrate and rich in fat, with moderate protein, is more effective in reducing the production of insulin by the pancreas. Suprarenal extract by mouth was given in three cases as an adjuvant (19), but it did not appear to offer much advantage over diet alone. Thyroid has been tried without benefit (23).

For emergency treatment during an attack, epinephrine administered hypodermically and glucose by mouth constitute two potent therapeutic measures. Both act promptly. While glucose has a more lasting effect, epinephrine possesses

the advantage of being administered easily during coma or convulsions which may render the ingestion of glucose impossible. It is important to remember that glucose per rectum is ineffective. The effect of levulose is doubtful, and presumably that of galactose as well. Pituitrin, pitressin and ephedrine are unreliable.

When the symptoms persist and increase, surgical attack on the pancreas becomes the treatment of choice. In all cases in which an adenoma has been found at operation and removed, relief from symptoms has been complete. Of the 15 cases without demonstrable tumor (25) in which a partial resection of the pancreas was carried out, improvement was noticed in 5. These were instances in which a large portion of the gland was removed. Graham and Hartmann (9) and more recently McCaughan (17) have advocated subtotal pancreatectomy for cases of hyperinsulinism in which a tumor is not demonstrable. It seems reasonable to believe that, as in the case of the thyroid, satisfactory results may not be obtained unless a subtotal excision of the gland is carried out.

It does not seem necessary to go into the details of surgical technic, as these are available elsewhere (8, 17, 25). It should, however, be emphasized that exposure must be adequate and exploration of the pancreas thorough, as multiple adenomata were found in four of the seventeen cases reported previously (8, 25). In our case a long left rectus incision and division of the gastrocolic omentum allowed satisfactory access to the gland. Others have preferred to use a transverse incision (25) or to approach the pancreas by the supragastric route (9). In instances in which mobilization or partial resection of the pancreas is attempted and serious difficulty is encountered due to hemorrhage from branches of the splenic vessels, we would like to suggest simple ligation of the splenic artery near its point of origin as a satisfactory means of controlling the flow of blood. Experience with this procedure as a substitute for splenectomy in certain instances has convinced us that ligation of this vessel is by no means an indication for subsequent removal of the spleen.

## SUMMARY

A case of chronic postabsorptive hypoglycemia associated with coma and convulsive seizures is reported. On surgical exploration a small tumor of the pancreas was found, the excision of which led to complete relief of the symptoms. The tumor was composed of cells of the islands of Langerhans, and yielded considerably more insulin than normal pancreatic tissue. Detailed studies of the blood sugar and metabolism before and after operation are presented. Evidence is adduced showing that the hypoglycemia was, in a large measure, related to the fact that the combustion of carbohydrate proceeded at a faster rate and played a more dominating rôle in energy metabolism than is normal, thus sparing fat and protein. This action, combined with overeating, produced obesity. Glucose by mouth and adrenalin hypodermically were promptly efficacious in combating the hypoglycemia. Pituitrin, pitressin, ephedrine, levulose by mouth and glucose per rectum were ineffective.

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# THE VOLUME OF THE EXTRACELLULAR FLUIDS OF THE BODY<sup>1</sup>

By PAUL H. LAVIETES, JACQUES BOURDILLON AND KALMEN A. KLINGHOFFER

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Changes in the volume of extracellular fluids of the body can be calculated from the exchanges of sodium (1) or of chloride (2), appropriate corrections being made when necessary for changes of concentration of these ions in the fluids (2). Such studies are technically difficult and time-consuming and yield no absolute values for volume of extracellular fluids. A diffusible, non-metabolized substance not normally present in the body but which, like sodium and chloride, is restricted to extracellular fluids would afford a convenient measure of the absolute volume of these fluids at any time. The experiments which follow represent an attempt to find such a substance.

Sucrose injected intravenously was tried first because it was known that this substance traverses capillary membranes with ease and yet does not enter red blood corpuscles. Later, the results of experiments of Crandall and Anderson (3) on the distribution of sulfocyanate suggested that this substance might be of use. Still later, the distribution of sulfate was studied when it was noted from the data of earlier experiments following the intravenous administration of  $\text{Na}_2\text{SO}_4$  to dogs (4) that sulfate apparently distributes itself through only about 20 per cent of the body by weight.

## EXPERIMENTAL SUBJECTS AND PLAN OF EXPERIMENTS

The subjects for the experiments were normal male laboratory workers, convalescent patients without evident abnormalities of hydration, and four patients with advanced renal failure without clinical evidence of edema. All experiments were started in the postabsorptive state, but in some instances small amounts of water, fruit juice and coffee were given during the experiments.

Sucrose, sodium sulfate and sodium sulfocyanate were injected intravenously, in doses of 15

to 30 grams for sucrose, 19 to 65 milliequivalents for sodium sulfate, and 0.8 to 1.8 grams for sodium sulfocyanate. Potassium sulfocyanate in doses of 1.25 to 1.75 grams has also been used *perorally*.

KSCN was given quantitatively *per os* as a 2.5 per cent solution. No significant changes in blood pressure were produced in either normal or hypertensive subjects. The substances for intravenous administration were weighed into a beaker, made up with freshly distilled water (approximately 25 cc. per gram for  $\text{NaSCN}$  and  $\text{Na}_2\text{SO}_4$  and 5 cc. per gram for sucrose), and sterilized by boiling. Merck's reagent grade anhydrous  $\text{Na}_2\text{SO}_4$  and commercial granulated sucrose required no preliminary desiccation, but  $\text{NaSCN}$  was dried to constant weight at 100° C. Injections were made at the rate of 10 cc. per minute. No toxic effects were noted, and diuresis was not provoked. The amount of solute left in the beaker and syringe was determined by analysis and correction made. Blood and urine samples were taken at varying intervals after the injections, and serum was separated from the blood after it was allowed to clot under oil.

## ANALYTICAL METHODS

1. *Sucrose.* The iodometric titration of Shaffer and Somogyi (5) using "reagent 50" with 5 grams KI and 0.488 gram  $\text{KH}(\text{IO}_3)_2$  per liter was used before and after hydrolysis.

For serum the Somogyi filtrate was used (6). Urine was merely diluted with water to contain between 15 and 30 mgm. of sucrose per 100 cc. Hydrolysis was effected by heating at 85 to 100° C. for two hours, in a 25 cc. volumetric flask covered with tinfoil, 20 cc. of serum filtrate or diluted urine plus 1.2 cc. 0.1 N HCl. After cooling, 0.1 N NaOH was added until the full blue color was obtained with bromcresol green. This usually required almost exactly 1.2 cc. of the alkali, and a drop in excess was found to be without effect on the subsequent reduction. The whole was made

<sup>1</sup> Part of the expense of this investigation was defrayed by a grant from the Ella Sachs Plotz Foundation.

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TABLE I

*The volume of distribution of sucrose in normal man*

Experiment number	Subject	Amount injected	Time after injection	Amount left in body	Concentration in serum	Volume of distribution of sucrose	
		grams	hours	grams	mgm.	kgm.	per cent of body weight
1	K	16.2	.5	11.6	112	10.4	16.3
			1.0	9.3	82	11.3	17.7
			1.5	7.6	73	10.4	16.3
2	K	21.1	1.0	12.3	117	10.5	16.4
			2.0	8.5	71	12.0	18.8
3	K	21.2	1.0	11.4	136	8.4	13.1
			1.5	8.9	86	10.4	16.3
			2.0	7.0	67	10.5	16.4
4	P	15.0	1.0	7.8	57	13.7	22.1
			2.0	5.1	41	12.4	20.1
5	L	20.7	1.0	12.2	85	14.3	17.0
			2.0	8.3	56	14.8	17.6
6	L	18.7	1.0	10.7	65	16.5	19.7
			2.0	7.5	43	17.4	20.7
7	L	17.4	1.0	11.0	67	16.4	19.5
			2.0	6.2	36	17.2	20.5
8	L	30.0	1.0	19.6	115	17.0	20.2
			2.0	14.3	76	18.8	22.5
			3.0	10.6	58	18.3	21.8
9	R	19.2	1.0	11.2	85	13.2	22.8
			2.0	8.1	53	15.3	26.4
10	R	19.9	1.0	12.9	108	11.9	20.5
			2.0	8.6	57	15.1	26.0
11	R	18.6	1.0	11.4	82	13.9	24.0
			2.0	7.9	53	14.9	25.7
12	S	18.0	1.0	10.5	62	16.9	29.2
			2	6.8	42	16.2	28.0

3 subjects were normal, young male physicians. Subject R was a rather undernourished elderly man with a moderate degree of arteriosclerosis who entered the hospital for treatment of Me-niere's syndrome. Subject S was a young man recently recovered from an attack of catarrhal jaundice. The reproducibility of the results in the subjects in which more than one determination was made is so good that the variability for different subjects must be accepted as real. The series is too small to attempt to derive an average value for normal subjects.

The results of the experiments with SCN in normal subjects are recorded in Table II. All the subjects were young male physicians with

TABLE II

*The volume of distribution of SCN in normal man*

Experiment number	Subject	Amount given	Time after administration	Amount left in body	Concentration in serum	Volume of distribution of SCN	
		mgm.	hours	mgm.	mgm.	kgm.	per cent of body weight
1	L	1250	3.00	1244	6.4	19.4	23.1
			8.00	1219	6.4	19.1	22.8
			24.00	948	4.9	19.3	23.0
2	L	1240	10.00	1187	6.2	19.1	22.8
3	L	1250	5.00	1182	6.2	19.1	22.8
4	B	1250	10.00	1212	7.3	16.6	25.9
5	B	1750	10.00	1695	10.0	17.0	26.3
6	W	1250	10.00	1197	7.1	16.9	20.1
7	K	1250	2.00	1240	8.0	15.5	24.2
8	Pe	1500	3.00	1234	8.1	15.3	23.9
			10.00	1485	7.4	20.1	28.3
			1.00	1782	9.8	18.2	21.8
9	L	1800	2.00	1770	9.7	18.3	21.9
			3.00	1757	9.6	18.3	21.9
			.10	1237	8.9	13.9	16.6
10	L	1238	.60	1236	7.3	16.9	20.1
			1.50	1240	8.5	14.6	20.6
			2.75	1232	8.2	15.0	21.2
11	Sti	1254					

the exception of the last who was a young woman with fully compensated rheumatic heart disease, and the subject of Experiment 8 who had mild diabetes without evidence of any other abnormality. In the first 8 experiments KSCN was given by mouth, in most instances on the evening preceding the study. In the last 3 experiments NaSCN was injected intravenously. The distribution of SCN was apparently completed within 3 hours after the peroral administration of KSCN in Experiment 1, and within 1 hour after the intravenous administration of NaSCN in Experiment 9. In Experiment 10, the distribution was apparently incomplete in 35 minutes, judging from the relation of the value obtained at that time to other determinations on the same subject which agree remarkably well with one another, averaging 22.9 per cent of the body weight for the early experiments done with KSCN and 21.9 per cent for the experiment done 2 months later with NaSCN. In the third experiment the recorded concentration of KSCN in serum is actually the difference between the original level of 2.2 mgm. and the final one of 8.4 mgm. In all the other experiments, the serum was free from SCN at the start. The two determinations on Subject B, done at an interval of 6 months, give values of 25.9 and 26.3 per cent of the body

up to 25 cc., and 5 cc. aliquots were taken for the copper reduction. At the same time 4 cc. of untreated serum filtrate plus 1 cc. of water was used for the determination of glucose. Serum filtrate containing glucose without sucrose gave identical results before and after being subjected to the above hydrolysis.

From the analysis of solutions of sucrose containing 5 to 30 mgm. per 100 cc., a curve, which proved to be rectilinear, was constructed relating sucrose concentrations to thiosulfate. From this curve, serum sucrose was estimated from the difference between the titrations before and after hydrolysis. The accuracy of the sucrose determination is within  $\pm 2$  per cent for concentrations greater than 50 mgm. per 100 cc. of serum.

2. *Sulfocyanate*. Color was developed in a trichloroacetic acid filtrate of serum by the addition of the ferric nitrate reagent described by Crandall and Anderson (3). This is made by diluting 50 grams  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  and 25 cc.  $\text{HNO}_3$  to a liter with water.

One cubic centimeter of serum was used for each of duplicate determinations. The precipitation of proteins was done in a round-bottomed tube ( $1.5 \times 10.0$  cm.), adding 20 per cent trichloroacetic acid drop by drop with vigorous shaking until a homogeneous mixture was obtained (usually after 6 or 8 drops have been added), after which the remainder of the 1 cc. of acid was added more rapidly. The tube was then stoppered, shaken vigorously, and centrifuged after standing at least 10 minutes. One cubic centimeter of the supernatant fluid was transferred to another tube in which color was developed with an equal volume of ferric nitrate reagent. Comparison with an aqueous standard solution of SCN plus reagent was made in a microcolorimeter. Omission of trichloroacetic acid from the standard was found to introduce no error. Since the color fades on standing, it must be developed in the standard and unknown at the same time and read within 10 minutes. In protein-free urine, no preliminary treatment is necessary, but SCN-free urine of the same color as the unknown was added to the standard and an equal amount of water to the unknown.

Duplicate determinations agree within  $\pm 3$  per cent, usually within  $\pm 1$  per cent.  $\text{NaSCN}$

added to serum and transudates in an amount equivalent to 10 mgm. per cent has been recovered repeatedly with an error of  $\pm 1$  per cent. Serum has been left in stoppered tubes at room temperature for 48 hours without demonstrable change in the sulfocyanate content.

3. *Sulfate*. The alkalimetric benzidine titration of Cope (7) as modified by Bourdillon (8) was used.

## RESULTS

The volume of body fluid through which each solute is distributed is calculated by dividing the amount left in the body at the time of blood sampling by its concentration in the serum. The implied assumption that the concentration of the solute in the serum is the same as that in the other fluids through which it diffuses is of course incorrect, but for reasons to be discussed later only this rough method of calculation will be used at present. If the substance is present in the serum before the injection, as is true of  $\text{SO}_4$  always and SCN occasionally when it has been used within the preceding week, the change of concentration in the serum is used. This has been found satisfactory in normal subjects given SCN repeatedly, and concentrations in the serum of 25 mgm. per cent have been attained without deleterious effect.

The significant data for the experiments with sucrose and the volumes of distribution calculated from the data are recorded in the first table. In general the calculated volume is significantly higher 2 hours after the injection than it is an hour earlier. The few studies in which determinations were done at one-half hour intervals indicate that diffusion is as complete one and one-half hours after the injection as it is one-half hour later. Studies after the third hour in normal subjects are impractical because of the rapidity of excretion of sucrose. In subjects with marked renal impairment without edema, however, it has been possible to show that no further distribution of sucrose occurs three and four hours after the injection. If only results obtained one and one-half or two hours after the injections are accepted, the average volume of distribution for Subject K is 17.2 per cent of the body weight; for Subject P, 20.1 per cent; for Subject L, 20.3 per cent; for Subject R, 26.0 per cent, and for Subject S, 28.0 per cent. The first

TABLE I

*The volume of distribution of sacrose in normal man*

Experiment number	Subject	Amount injected	Time after injection	Amount left in body	Concentration in serum	Volume of distribution of sacrose	
		grams	hours	grams	mgm.	kgm.	per cent of body weight
1	K	16.2	.5	11.6	112	10.4	16.3
			1.0	9.3	82	11.3	17.7
			1.5	7.6	73	16.4	16.3
2	K	21.1	1.0	12.3	117	10.5	16.4
			2.0	8.5	71	12.0	18.8
3	K	21.2	1.0	11.4	136	8.4	13.1
			1.5	8.9	86	10.4	16.3
			2.0	7.0	67	10.5	16.4
4	P	15.0	1.0	7.8	57	13.7	22.1
			2.0	5.1	41	12.4	20.1
5	L	20.7	1.0	12.2	85	14.3	17.0
			2.0	8.3	56	14.8	17.6
6	L	18.7	1.0	10.7	65	16.5	19.7
			2.0	7.5	43	17.4	20.7
7	L	17.4	1.0	11.0	67	16.4	19.5
			2.0	6.2	36	17.2	20.5
8	L	30.0	1.0	19.6	115	17.0	20.2
			2.0	14.3	76	18.8	22.5
			3.0	10.6	58	18.3	21.8
9	R	19.2	1.0	11.2	85	13.2	22.8
			2.0	8.1	53	15.3	26.4
10	R	19.9	1.0	12.9	108	11.9	20.5
			2.0	8.6	57	15.1	26.0
11	R	18.6	1.0	11.4	82	13.9	24.0
			2.0	7.9	53	14.9	25.7
12	S	18.0	1.0	10.5	62	16.9	29.2
			2	6.8	42	16.2	28.0

3 subjects were normal, young male physicians. Subject R was a rather undernourished elderly man with a moderate degree of arteriosclerosis who entered the hospital for treatment of Meniere's syndrome. Subject S was a young man recently recovered from an attack of catarrhal jaundice. The reproducibility of the results in the subjects in which more than one determination was made is so good that the variability for different subjects must be accepted as real. The series is too small to attempt to derive an average value for normal subjects.

The results of the experiments with SCN in normal subjects are recorded in Table II. All the subjects were young male physicians with

TABLE II

*The volume of distribution of SCN in normal man*

Experiment number	Subject	Amount given	Time after administration	Amount left in body	Concentration in serum	Volume of distribution of SCN	
		mgm.	hours	mgm.	mgm.	kgm.	per cent of body weight
1	L	1250	3.00	1244	6.4	19.4	23.1
			8.00	1219	6.4	19.1	22.8
			24.00	948	4.9	19.3	23.0
2	L	1240	10.00	1187	6.2	19.1	22.8
3	L	1250	5.00	1182	6.2	19.1	22.8
4	B	1250	10.00	1212	7.3	16.6	25.9
5	B	1750	10.00	1695	10.0	17.0	26.3
6	W	1250	10.00	1197	7.1	16.9	20.1
7	K	1250	2.00	1240	8.0	15.5	24.2
			3.00	1234	8.1	15.3	23.9
8	Pe	1500	10.00	1485	7.4	20.1	28.3
9	L	1800	1.00	1782	9.8	18.2	21.8
			2.00	1770	9.7	18.3	21.9
			3.00	1757	9.6	18.3	21.9
10	L	1238	.10	1237	8.9	13.9	16.6
11	Sti	1254	.60	1236	7.3	16.9	20.1
			1.50	1240	8.5	14.6	20.6
			2.75	1232	8.2	15.0	21.2

the exception of the last who was a young woman with fully compensated rheumatic heart disease, and the subject of Experiment 8 who had mild diabetes without evidence of any other abnormality. In the first 8 experiments KSCN was given by mouth, in most instances on the evening preceding the study. In the last 3 experiments NaSCN was injected intravenously. The distribution of SCN was apparently completed within 3 hours after the peroral administration of KSCN in Experiment 1, and within 1 hour after the intravenous administration of NaSCN in Experiment 9. In Experiment 10, the distribution was apparently incomplete in 35 minutes, judging from the relation of the value obtained at that time to other determinations on the same subject which agree remarkably well with one another, averaging 22.9 per cent of the body weight for the early experiments done with KSCN and 21.9 per cent for the experiment done 2 months later with NaSCN. In the third experiment the recorded concentration of KSCN in serum is actually the difference between the original level of 2.2 mgm. and the final one of 8.4 mgm. In all the other experiments, the serum was free from SCN at the start. The two determinations on Subject B, done at an interval of 6 months, give values of 25.9 and 26.3 per cent of the body

weight respectively. The body weight at the time of the second study exceeded the earlier one by 1 kgm. The calculated volumes for Subjects B and W, with body weights of 64 and 84 kgm. respectively, were almost identical. This is probably related to the fact that the former was quite sparsely covered with fat while the latter was moderately obese. The values for this small series, ranging from 20.1 to 28.3 per cent of the body weight, do not permit the deduction of any significant average.

TABLE III

*The volume of distribution of  $\text{SO}_4$  in normal man*

Experi- ment num- ber	Sub- ject	Amount injected	Time after injection	Amount left in body	Concen- tration in serum	Volume of di- stribution of $\text{SO}_4$	
		m.eq.	hours	m.eq.	m.eq.	kgm.	per cent of body weight
1	L	47.3	.50	33	2.00	16.5	19.7
			2.25	18	0.96	18.7	22.3
2	L	41.8	.10	35	2.98	11.8	14.1
			.60	26	1.70	15.3	18.2
3	B	29.9	.25	23	1.98	11.6	17.8
			.75	17	1.22	13.9	21.4
			1.75	13	0.69	18.8	28.9
4	We	50.7	.50	40	4.28	9.3	17.2
			2.00	15	1.36	11.0	20.8

In the sulfate experiments, recorded in Table III, an endogenous excretion of 1 m.eq. per hour has been assumed (9). The recorded concentrations of sulfate in serum are actually the differences between the fasting levels and the levels at the times indicated. In Experiments 1, 3 and 4 where values are available 2 hours after the injections, the volumes of distribution are of the order of magnitude obtained with sucrose and SCN. To be more specific, Subject B shows a value of 28.9 per cent of the body weight as compared with 26.1 per cent with SCN; and Subject L, a value of 22.3 per cent as compared with averages of 22.4 per cent with SCN and 20.3 per cent with sucrose.

The results of some studies in which both sucrose and SCN were administered are given in Table IV. The results of Experiment 1 confirm the previous suggestion that while the distribution of sucrose is completed only during the second hour after injection in normal subjects, SCN is distributed more rapidly. The subject for Experiments 2 and 3 had advanced renal failure with-

TABLE IV

*Studies after the administration of both sucrose and SCN*

Experi- ment num- ber	Sub- ject	Time after adminis- tration	Volume of distribution			
			Sucrose	SCN	Sucrose	SCN
		hours	kgm.	kgm.	per cent of body weight	per cent of body weight
1	L	1.00	17.0	18.1	20.2	21.6
		2.00	18.8	18.2	22.4	21.6
		3.00	18.3	18.4	21.8	21.9
2	D	1.00	17.5	20.7	35.3	41.8
		2.00	18.3	19.0	36.9	38.4
3	D	1.66	15.3	17.4	30.0	34.1

out edema. Between Experiments 2 and 3 salt intake was restricted for 3 days while water was given in large amounts. The body weight fell 0.6 kgm. and the base concentration in the serum fell more than 5 m.eq. Extracellular fluid was presumably sacrificed by transfer of water to the cells in order to lower their osmotic pressure to that of their environment as well as by absolute loss from the body indicated by the weight change. The values calculated from the distribution of sucrose and SCN parallel the expected fall in volume of the extracellular fluids. It was shown that when large amounts of  $\text{Na}_2\text{SO}_4$  were injected intravenously into subjects who were given KSCN *per os* on the previous night, the concentrations of SCN and of Cl fell proportionately (8). Since excretion of these ions in the urine was negligible during the injections, this could only mean that Cl and SCN were distributed through approximately the same fraction of the body fluids. Since this fraction expands as the result of injection of a sodium salt, it is probably the extracellular fraction.

Preliminary studies on the distribution of the substances used between serum and blood cells and serum and transudates have been made. Previous work showing that neither inorganic sulfate nor sucrose added to blood *in vitro* permeates red blood cells has been confirmed. In one experiment when KSCN was added to oxalated whole blood *in vitro* in concentration of 20 mgm. per 100 cc. without precaution against loss of  $\text{CO}_2$ , the concentration in the plasma was found to be 23.8 mgm. per 100 cc. The concentration in cells, then, must have been somewhat lower than in plasma. No more exact knowledge concerning

the distribution of SCN between serum and cells is available at present. The ratio of sulfate in transudates to sulfate in serum has been found to be approximately unity (8). On two occasions serum and transudates obtained from patients after the injection of sucrose contained sucrose in approximately the same concentration. Exact agreement was not expected because the rapidity with which sucrose is excreted prevented the allowance of a sufficient interval between the injection and the study to insure the attainment of diffusion equilibrium. Studies made 12 to 64 hours after the administration of SCN to patients with transudates show an average concentration in serum ten per cent higher than that in transudates. With two exceptions, the concentration in serum exceeds that in fluid by between 7 and 14 per cent, with an average of 10 per cent. In the exceptions, the excesses were 5 and 21 per cent respectively. Two patients in the series who were deeply jaundiced gave ratios similar to those for the rest. That the difference between SCN concentration in serum and fluids can not be attributed to insufficient time for complete diffusion has been shown by the fact that the difference was the same in repeated determinations on the same individual made from 12 to 64 hours after giving the SCN, and also by the fact that in one instance the concentration of SCN in ascitic fluid and serum taken from this patient remained unchanged after equilibration for 48 hours across a cellophane membrane. That the observed differences are not due to analytical error has been shown by the quantitative recovery from SCN-free serum and transudates of added SCN in amounts equivalent to 10 mgm. per cent. Sucrose and SCN gain access to spinal fluid in traces only (3, 10). This is probably another evidence that spinal fluid is a highly specialized fluid not comparable to transudates and need not deduct from the value of these substances in measuring extracellular fluids.

Sucrose has been quantitatively recovered from the urine within 24 hours after intravenous injection in normal subjects on 3 occasions. Within 5 days of the administration of 1250 mgm. of KSCN *per os* to a normal subject, 1196 mgm. were recovered from the urine, the excretion on the fifth day being 56 mgm. This is in accord with the finding of Pollak (11) that SCN given to animals either *per os* or parenterally can be re-

covered quantitatively from the urine. In the six and one-half hours following the injection of 47.3 m.eq. of  $\text{SO}_4$  into a normal subject 51.7 m.eq. of inorganic  $\text{SO}_4$  appeared in the urine. Allowing 1 m.eq. per hour for endogenous production, this indicates satisfactory quantitative recovery.

#### DISCUSSION

Sucrose, SCN and inorganic  $\text{SO}_4$  are apparently distributed through approximately 20 per cent of the weight of normal man. This portion, then, must differ from the remainder of the water of the body. That it probably represents extracellular fluids seems probable from the fact that it varies in size with procedures calculated to change the volume of the extracellular fluids. An entirely extracellular distribution for sucrose and inorganic  $\text{SO}_4$  can be accepted with little reservation since these substances do not enter blood cells although they pass freely into transudates. The fact that the distribution of SCN in the body follows so closely that of these substances under varying conditions and in different subjects is presumptive evidence that SCN too remains entirely without the cells if the red corpuscles of the blood are excepted. Additional evidence to support this contention is presented in Table V in which analyses from Corper (12) of SCN in blood and tis-

TABLE V

*Comparison of tissue analyses and blood analyses for Cl (Cameron and Walton) and for SCN (Corper)*

	Cl		SCN	
	Grams per kgm.	Tissue blood	Grams per kgm.	Tissue blood
Blood.....	3.00		0.64	
Lung.....	2.30	0.77	0.52	0.81
Kidney.....	2.51	0.84	0.34	0.53
Heart.....	1.19	0.40	0.39	0.61
Liver.....	1.36	0.45	0.27	0.42
Muscle.....	0.67	0.22	0.08	0.13

sues of dogs 24 to 72 hours after the injection of NaSCN are compared with analyses from Cameron and Walton (13) of Cl in blood and tissues of dogs. Chloride in the blood of dogs has been assumed at 300 mgm. per 100 cc. The concentrations of SCN, like those of Cl, vary with the vascularity of the organ, and the ratios of SCN



in tissues to SCN in blood are of the same order of magnitude as the corresponding ratios for Cl. If it is admitted that Cl need enter no cells other than blood cells, then the same may be said of SCN.

Of the three substances employed SCN is distributed through the body most rapidly, sucrose least rapidly. The excretion of SCN is many times slower than that of either  $\text{SO}_4$  or sucrose. The rapidity of excretion of the latter two together with their relatively slow distribution make their use impractical in patients with excessive transudation, particularly into the serous cavities, unless renal function is much impaired. On the contrary SCN is excreted so slowly that sufficient time may be allowed between the administration of the substance and the study of its distribution to insure complete diffusion through the largest accumulations of fluids. The occurrence of any appreciable gradient between serum and extravascular fluids with such slow excretion is inconceivable. SCN has the further advantages that it may be given by mouth as a K salt thus avoiding the administration of Na to edematous patients, and presumably, since K is not retained in the serum, having no effect on total base or volume of extracellular fluids. The determination may be done with sufficient accuracy on small amounts of serum containing less than 1 m.eq. of SCN per liter, and if repeated determinations are necessary within 24 hours sufficient SCN remains in the body to obviate the administration of any more of the substance. Sucrose has the advantage that, being a non-electrolyte, it probably attains the same concentration in the water of serum and extravascular extracellular fluids. It has the disadvantages, however, that the analyses are time consuming and require concentrations in the serum of a magnitude which may change the volume of extracellular fluids appreciably. Furthermore, its rapid excretion in concentrated solution introduces possible error attendant upon the presence of considerable amounts of sucrose in the renal pelvis and possibly in incompletely emptied urinary bladders which in the calculation would be attributed to the extracellular fluids. For  $\text{SO}_4$ , the same disadvantages obtain with the additional factors that the exact distribution of this ion between serum and transudates is still uncertain and that in order to obtain concentra-

tions in the serum high enough for accurate measurement, considerable amounts of a Na salt must be given.

For practical purposes as a measure of the volume of extracellular fluids, then,  $\text{SO}_4$  may be eliminated from consideration for the present. Sucrose is suitable for subjects with impaired renal function and, in the absence of abnormal transudates, for subjects with normal renal function. For general application, SCN affords the greatest number of advantages. In the light of our present knowledge the most accurate representation of extracellular fluid volume from the distribution of SCN would be as follows:

$$\frac{\text{Amount retained} - \text{Amount in blood}}{\text{Concentration in transudates}} + \text{Serum volume.}$$

Since SCN is present in red blood cells in approximately the same concentration as in serum, the amount of SCN in blood is roughly the product of blood volume and concentration in serum. This is of course only roughly true, as has been pointed out before. In the one experiment in which the relationship was studied, the concentration in cells was slightly lower than that in serum. If the actual concentration of SCN in transudates is not available, it may be estimated as 100/110 of the concentration in serum or determined directly by ultrafiltration of serum.<sup>1</sup> For sucrose, since its concentration in the water of all extracellular fluids is probably uniform, the amount retained in the body divided by the concentration of sucrose in the water of serum should give the volume of extracellular fluids directly without consideration of blood or serum volumes.

TABLE VI

*Calculations of volume of extracellular fluid for Experiment 1, Table IV*

Hours after injection	Sucrose			SCN					Volume of distribution	
	Left in body	Concentration in water of serum	Volume of extracellular fluids	Left in body	Concentration		Amount in blood	Volume of extracellular fluids	Sucrose	SCN
					In serum	In transudates				
	grams	grams per liter	liters	mgm.	mgm. per liter	mgm. per liter	mgm.	liters	liters	liters
1	15.6	1.23	15.9	1752	99	83	721	16.3	17.0	18.1
2	14.2	0.82	17.4	1770	97	87	775	14.4	18.8	18.2
3	16.6	0.62	17.1	1737	90	86	764	16.6	18.2	18.4

These calculations for volume of extracellular fluids have been applied to Experiment 1 of Table IV, in which sucrose and sulfocyanate were injected simultaneously. The subject, one of the authors, weighing 84 kgm., was assumed to have 8 liters of blood with 5 liters of serum. The data for and the results of the calculations are presented in Table VI, and the "volumes of distribution" of these substances as previously calculated by dividing the amount retained by the concentration in the serum are given for comparison. Volume of extracellular fluid calculated from SCN and sucrose are in good agreement. While the differences between these values and the "volumes of distribution" are not striking, these differences will vary widely with the changes in the relation of blood volume to total extracellular fluid volume which occur in disease. Any accurate calculation of extracellular fluid volume from the distribution of SCN must ultimately take account of the differences in the concentration of the substance in blood and extravascular fluids. Sucrose has the advantage that this differentiation presumably does not exist.

The distribution of SCN between serum and transudates is contrary to that of other anions and is at direct variance with the Donnan theory if all the SCN is diffusible. It seems probable that some of the SCN in the serum is restrained in some manner, possibly in combination with proteins. The combination of anions with protein at body reaction has been demonstrated for  $\text{CO}_2$  and hemoglobin (14). The diffusion of SCN from serum is being subjected to further study.

Crandall and Anderson (3) showed that SCN enters all the gastro-intestinal secretions, attaining concentrations of the same order of magnitude as it does in serum. Sucrose does not enter the gastro-intestinal tract. In the present studies, done in the postabsorptive state and in the absence of digestive disturbances, the amount of SCN in the gastro-intestinal tract must be very small. It may prove possible with sufficient refinement of the methods to determine the amount of fluid present in the gastro-intestinal tract in

disease by simultaneous studies with SCN and sucrose.

In experiments on 4 subjects with advanced renal insufficiency without edema (one of which is recorded in Table IV) the calculated volume for extracellular fluid was extremely high, making up 30 to 43 per cent of the body weight. The consistently high values obtained in these subjects probably indicate an extreme degree of cellular wastage with replacement by interstitial fluid.

#### SUMMARY AND CONCLUSIONS

Sucrose, SCN and inorganic  $\text{SO}_4$ , are distributed through approximately the same fraction of the body fluids.

This fraction varies in magnitude with measures planned to vary the extracellular fluid volume and probably is composed of the extracellular fluids only.

It makes up approximately 20 per cent of the weight of normal subjects, although there is considerable individual variability. Extremely high values have been found in cases of terminal nephritis without edema, probably indicating marked cellular wastage.

SCN is distributed more rapidly than  $\text{SO}_4$  or sucrose.

The speed of excretion of  $\text{SO}_4$  and sucrose interferes with their practical application to the measurement of extracellular fluid volume. Sucrose has the advantage, however, that being a non-electrolyte, it is probably present in the same concentration in the water of serum and that of extravascular extracellular fluids.

SCN has numerous advantages. It enters red blood cells, however, and is present in serum partly in bound form. In order to use it as a practical method of measuring volume of extracellular fluids, the concentration of SCN in transudates or ultrafiltrate of serum must be known. It has been shown that the concentration in transudates is approximately 100/110 that in serum. Until more accurate information is available, a tentative method for the calculation of extracellular fluid volume from data obtained after the administration of SCN is:

$$\frac{\text{Amount retained in body} - (\text{concentration in serum} \times \text{blood volume})}{100/110 \times \text{concentration in serum}} \div \text{Serum volume.}$$

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STUDIES OF THE VARIATIONS IN THE ANTISTREPTOLYSIN TITER OF THE  
BLOOD SERUM FROM PATIENTS WITH HEMORRHAGIC NEPHRITIS. I.  
CONTROL OBSERVATIONS ON HEALTHY INDIVIDUALS AND  
PATIENTS SUFFERING FROM DISEASES OTHER  
THAN STREPTOCOCCAL INFECTIONS

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During some studies which have been in progress for several years upon the etiology and the clinical course of acute glomerular nephritis, it was considered desirable to examine the serum from these patients for antibodies to hemolytic streptococci. Comment has frequently been made to the effect that infections by this organism involving the upper respiratory tract usually precede the onset of acute hemorrhagic nephritis, and particular attention has been drawn to this matter in previous publications (1). Efforts to demonstrate the presence of specific agglutinins and specific precipitins for hemolytic streptococci in the serum from cases of acute, subacute and chronic glomerular nephritis were met with indifferent success and very irregular results, and the investigations were being abandoned when Todd published his observations on antistreptolysin.

Todd (2) showed that the hemolysin produced by hemolytic streptococci, grown in a special medium free of animal sera, was antigenic and gave rise in rabbits to an antistreptolysin of considerable potency. Streptolysin formed by the growth of hemolytic streptococci in serum broth did not excite antibodies capable of neutralizing serum broth streptolysin nor would antistreptolysin of high titer neutralize serum broth streptolysin. Since it seemed probable that antistreptolysin might be formed in the serum of animals immunized to hemolytic streptococci, Todd tested scarlatinal antitoxin, erysipelas antitoxin and puerperal septicemia antitoxin, and found that antistreptolysin was present in high titer. Whereas the minimal neutralizing dose of normal horse serum was 0.2 cc. to 0.04 cc., the minimal neutralizing dose of the specific antitoxins was from 0.005 to 0.0002 cc. He further observed that the titers of diphtheria antitoxin, tetanus antitoxin, staphylococcus antitoxin, Small's non-hemolytic streptococcal antitoxin, antipneumococcal serum, antimeningococcal serum, and anti-gas gangrene serum did not differ from those of normal horse serum. Todd (3) then studied the reaction of the blood serum of man during infections by hemolytic streptococci. He recorded the antistreptolysin values of serum in units which are calculated as the reciprocal of the fractions of the cubic centimeters of serum employed. Under these conditions 0.01 cc. of serum equals 100 units, 0.03 cc. is equivalent

to 33.3 units and so forth. He found that the titers of sera from normal individuals varied between 20 units and 100 units. During convalescence from scarlet fever, erysipelas and tonsillitis, the antistreptolysin of the serum was elevated and sometimes reached 1,000 to 1,300 units. The serum from a limited number of patients suffering from infections that were not associated with hemolytic streptococcal infections gave titers of approximately normal values. During the acute phase of severe or fatal infections by hemolytic streptococcus the antihemolytic titers of the serum were not elevated. In some observations made upon the serum from nurses at the Presbyterian Hospital in New York, Todd found that during the period of normal health, or when persons were simply exposed to hemolytic streptococcal infections, that the antistreptolysin content of the serum was within normal limits. During the acute phase of streptococcal pharyngitis the titers still remained low, but during convalescence the titers rose and remained high for as long as one to three months after the infection. Todd extended his observations to rheumatic fever and found that the sera of patients in the quiescent stage gave titers that were within normal limits. During the active stage, however, the antistreptolysin content of the serum was much increased. This increase of antistreptolysin appeared to be dependent upon the preceding streptococcal infection of the respiratory tract, and did not vary with the severity of the attack of rheumatic fever. The antistreptolysin values of the serum often remained high for months during the attack of rheumatic fever and into the period of convalescence. Todd concluded that his observations afforded evidence that rheumatic fever was preceded by hemolytic streptococcal infections.

These observations on rheumatic fever were immediately confirmed and extended by Coburn and Pauli (4). They found that in the normal individual the neutralizing dose of serum was usually 0.02 cc. but might be as small as 0.01 cc. or 100 units, but rarely less. Following an attack of scarlet fever or erysipelas the titer rose from a normal figure to 0.003 or even as high as 0.0003 cc. in a case of erysipelas. In the acute rheumatic process the neutralizing dose of serum often reached 0.005 cc. or less. In inactive rheumatic fever the titers of the sera were usually within normal limits. The antistreptolysin titer of the serum remained normal during the infectious

<sup>1</sup> The titrations of antihemolysin were made with the technical assistance of Cyril Skala.

and quiescent period preceding the acute attack of rheumatic fever, but rose abruptly just before the appearance of rheumatic symptoms. During the first week of rheumatic fever it reached its highest level, and maintained this throughout the attack. They believed that their observations furnished strong evidence that the rheumatic attack was initiated by hemolytic streptococci. Since this first publication, Coburn and Pauli (5) have extended their studies and reference will be made to their results later on.

Since the appearance of the first papers by Todd and by Coburn and Pauli, a considerable number of articles have been published on the subject. Griffiths (6) has found high antistreptolysin in titers in all cases of acute rheumatic fever, and in a much smaller proportion of a variety of arthritic conditions. He considered 50 units as the normal. Myers and Keefer (7) give rather high figures for the normal, but since they modified the method of Todd in preparing streptolysin, and employed sheep red blood cells instead of the red blood cells from rabbits their figures can scarcely be compared with those published by others. They confirmed the preceding observations by finding high titers in proven hemolytic streptococcal infections, and comparatively high titers in acute rheumatic fever. They did not find an increase in the antistreptolysin content of the serum from patients with rheumatoid arthritis or other joint infections. They could not relate the antistreptolysin content of the serum to agglutinins for hemolytic streptococci or to the skin sensitivity to the nucleoprotein of the *Streptococcus scarlatinae*. The studies of Wilson, Wheeler and Leask (8) did not support the assumption that a rise in the antistreptolysin titer of the serum was conclusive evidence of streptococcal respiratory infection, and they stated that a rise in the antistreptolysin titer was not a necessary accompaniment of rheumatic fever in children.

Lippard and Johnson (9) have shown, however, that the antistreptolysin content of the serum of normal infants under 17 months of age is low, the average being 24 units and 55 per cent being 2 units or less. The average for children over 17 months of age was 66 units. In the infants there was little tendency for the antistreptolysin titer of the serum to rise following infections by hemolytic streptococci, whereas in older children the response was comparable to that observed in adults. It was further found (10) that the treatment of hemolytic streptococcal infections of infants and children by transfusions of blood or injections of streptococcal antisera was not accompanied by a significant increase in antistreptolysin substance in the blood of the recipient. Blair and Hallman (11) titrated antistreptolysin in the sera from a few normal individuals and from patients suffering from a variety of conditions. They concluded that 100 units is the upper limit of normal. The antistreptolysin titer was moderately increased in about one-third of the sera from 45 cases of rheumatoid arthritis, very high in the sera from 7 cases of streptococcal infection as well as in 15 out of 18 cases of acute rheumatic fever. They concluded that a high titer of antistreptolysin in

blood serum signifies specifically infection by hemolytic streptococci.

Owing to the fact that many observers consider that the presence of distinctly abnormal amounts of antistreptolysin (over 200 units) in the blood serum is a definite indication of recent or existing infection by hemolytic streptococci, the test has been employed by some to detect such an infection. Morales-Otero and Pomales-Lebrón (12) tested the antistreptolysin titer of the sera from 41 cases of recurrent lymphangitis in Porto Rico, and since they often found the titers elevated considerably above that of their normal controls, they suggest that this condition is due to a recurring infection by hemolytic streptococci. Seegal and Lyttle (13) have determined the antistreptolysin titers of the serum from 22 cases of acute glomerular nephritis. An infection of the throat or upper respiratory tract preceded the onset of nephritis in 21 of these cases and in 15 of them the infections were associated with hemolytic streptococci. The sera were examined 6 to 57 days after the onset of the acute nephritis. Twenty of the 22 sera showed an increase of antistreptolysin titers ranging from 125 to 1250 units. They considered that their data offered strong evidence to support the contention that acute glomerular nephritis is chiefly related to a preceding hemolytic streptococcal infection.

It is unnecessary to repeat in detail the method of making the medium and preparing streptolysin. The directions given by Swift and Hodge (14), who were kind enough to acquaint us with the technique before their publications, were followed precisely. Nor is it desirable to repeat the details of performing the test, for this has been accurately described by Coburn and Pauli (5), and their method has been strictly adhered to. Todd's original "Aronson" strain of hemolytic streptococcus, which was kindly sent to us by Drs. Swift and Hodge, has been the organism used. Complete hemolysis has usually been obtained with from 0.1 cc. to 0.08 cc. of streptolysin, after it had remained sealed under vaseline at 4° C. for 2 to 3 weeks. The hemolytic titer of each batch of hemolysin preserved in separate tubes has remained practically unchanged for months. A fresh tube of streptolysin has been used for each series of tests. Even though Hodge and Swift (15) have pointed out that the combining unit of streptolysin with serum may remain constant when the hemolytic dose varies somewhat, the hemolytic dose of each tube of streptolysin has been determined before the tests with serum were made. As a rule  $2\frac{1}{2}$  minimal hemolytic doses

proved to be the amount which gave a constant combining power. A 5 per cent suspension of freshly drawn rabbits' red blood cells washed 6 times in 0.85 per cent NaCl was used for the tests. Each batch of streptolysin was titrated carefully against a serum of known antistreptolysin content before it was employed for the tests. The first lots of tested serum were sent to us by Drs. Swift and Hodge. Later, Dr. Todd very kindly sent us immune serum, the titer of which was 20,000 units per cc. In each series of tests a serum of known low titer and a serum of known high titer has been used for control. For many months the serum sent by Dr. Todd was the one employed for the high titer control. The tests have been read shortly after removal from the water bath and again after standing for 18 hours at 4° C. Except in the rarest instances the readings coincided. When they did not coincide the test was repeated.

It seemed obvious that if a study of the antistreptolysin content of the serum from patients with hemorrhagic Bright's disease was to be of any value, one should in the first place examine samples of serum from the same patient at fairly frequent intervals, and in the second place make tests on the serum from a number of normal individuals and from patients suffering from a variety of diseases in order that a fair background for comparisons might be obtained.

To this end we have examined more than 1,716 samples of sera for the antistreptolysin content from 55 supposedly normal individuals and from 516 patients suffering from a variety of conditions. The observations, for clarity of discussion, can best be divided into two parts. The first part deals with the results obtained from the titration for antistreptolysin in the serum from (a) presumably normal individuals, (b) from patients suffering from such chronic diseases as diabetes mellitus and exophthalmic goiter, (c) from patients affected by various forms of anemia, leukemia and new growths, (d) from patients with chronic inactive rheumatic heart disease, (e) from patients with essential hypertension, hypertensive heart disease, chronic vascular nephritis and syphilitic aortitis, and finally from patients suffering from a variety of infectious diseases not associated with hemolytic streptococci.

In order to obtain a fair average of the natural antistreptolysin content of the serum, 84 samples from 55 supposedly normal individuals have been examined. The results are given in Table I. In each instance the highest titer is recorded. In 40 individuals, or in 74.5 per cent, the titer lay

TABLE I

*Antistreptolysin titers of the blood serum from 55 normal individuals and 84 patients suffering from chronic disease*

Diagnosis	Antistreptolysin titers (units per cc.)														Total number of patients	Number of patients with titers over 100 units
	10	11	12.5	10.7	20	25	33.3	60	100	111.1	125	112.0	100.7	200		
Normal			3	5	1	20	1	19	5			1			53	1
Hypertrophic and myxedema				1		2		4							7	0
Diabetes mellitus	1			2		1		4			1				9	1
Anemias, chronic leukemia, paroxysmal hemoglobinuria, Hodgkins carcinoma of lung, infectious mononucleosis					2	4		7				2			15	2
Chronic rheumatic heart disease						6		5	2		3	2			15	5
Hypertension, chronic vascular nephritis, syphilitic aortitis, arteriosclerotic heart disease			1	1		11	1	14	1		5		1		35	6
Total	1		4	11	1	44	2	33	8		9	1	5		137	15

between 25 and 50 units; in 3 it was 12.5 units, and in 5 it was 100 units. In only one person was it above 100 units. In this one individual, who was apparently normal, repeated cultures from the throat failed to show either beta hemolytic streptococci or the minute hemolytic streptococci described by Long and Bliss (16); the titer on two occasions over a period of 3 months was 142.9. Eight months after the last titration, the serum showed a titer of 100. These results are similar though perhaps somewhat more uniform than those obtained by Coburn and Pauli (5, I and VI) from a much larger series of individuals in New York. Seventy-five per cent of 146 persons showed titers of 100 or below, and the median titer for 176 persons in good health was 83 units.

Though the usual titer for antistreptolysin appeared to fall between 25 and 50 units for the adults in our series, a result that corresponds to the natural titer (5, I), it seemed desirable to determine whether variations might occur in a single individual's serum over a fairly long period dur-

ing which time he would probably contract several minor infections. The sera from five normal individuals and from one case of hypertension with chronic nephritis have been examined at intervals over periods varying from 7 months to one year. The results are given in Table II. It may be seen that the titers remain comparatively constant in spite of colds and attacks of bronchitis and, with the exception of Number 3 already referred to, they fall well within the limits of the natural antistreptolysin for the larger group.

TABLE II

*Antistreptolysin titers (units per cc.) of the blood serum from 6 individuals over a period of 7 to 13 months*

Time of determination	Patient 1†	Patient 2†	Patient 3†	Patient 4†	Patient 5†	Patient 6†
months						
1	50.0	33.3	142.9	50.0	16.7	25
2	50.0			50.0	16.7	
	50.0			50.0	25	
3	50.0		142.9	100	20	
4				50.0	Severe cold	
				50.0	20	
5	50.0	33.3		50.0		
6	50.0			33.3		
				50.0		
7	50.0	50.0		16.7		
	33.3					
8						50.0
9						50.0
						50.0
10						50.0
						50.0
						50.0
11			100			
12	50.0		100	33.3*	20	
				125	20	
13				125		
14				100		

† Comments: Patient 1. Normal. Occasional colds. 2. Normal. 3. Normal. Chronic pharyngitis—repeated cultures show no B. hemolytic streptococcus or minute B. hemolytic streptococcus. 4. Normal. Several severe colds; infection of finger at \* with B. hemolytic streptococcus. 5. Normal. Repeated severe colds. 6. Hypertension with chronic vascular nephritis.

The determinations of the antistreptolysin content of sera from all the patients suffering from the various chronic diseases may be considered together. The results are compared in Table I with the normal individuals. In each instance

the highest titer obtained is recorded. One hundred and fifty specimens of serum were examined from 84 patients. In several instances five or more collections of serum were made over a period of several weeks or months. The titers remained remarkably constant for each individual. In general the titers vary little from the natural antistreptolysin content of serum. In 50 patients, or a little less than 60 per cent, the titers fell between 25 and 50 units; in one case of diabetes the titer was very low for the normal adult—10 units. This corresponds to the very low titers found by Lippard and Johnson (9) in infants under 18 months of age. In 14 patients, or 16.6 per cent, the titers were slightly elevated above the natural content. There was no obvious explanation for this increase.

The infectious diseases that are included in Table III were typhoid fever, malaria, bacterial endocarditis, meningococcal meningitis and gonococcal arthritis, a miscellaneous group including tuberculosis, epidemic jaundice, bronchitis, periarteritis nodosa and vesicular stomatitis, and finally lobar pneumonia. There were 66 individuals in this group.

TABLE III

*Antistreptolysin titers of the blood sera from 66 patients suffering from various forms of acute infection*

Diagnosis	Antistreptolysin titer (units per cc.)														Total number of patients	Total number of sera	Number of patients with titers over 100 units
	12.5	14.9	16.7	20	25	33.3	50	100	111.1	125	142.9	166.7	200	250			
Typhoid.....					1		2	1	1			1			6	11	2
Malaria.....			2		1		3			1					7	8	1
Bacterial endocarditis.....								5	1	1				1			
Meningococcus meningitis, gonococcus arthritis.....															8	21	2
Miscellaneous, including tuberculosis, bronchitis, pyelitis.....			1		1		3			1		2			8	9	3
Pneumonia.....					2		8										
					8	1	0	2		1		2		1	13	20	3
											3				21	41	4
Total.....		3		13	1	30	4	1	4		8		2	66	113	15	

One hundred and thirteen specimens of serum were examined, and frequently several bleedings were made at intervals from the same patient. Table III gives the results of these examinations. In each instance the highest antistreptolysin titer obtained was recorded.

It will be seen that though a slightly larger



proportion of these patients (22.7 per cent) than those suffering from chronic diseases (16.6 per cent) gave titers of antistreptolysin above the normal level, only two patients, one an instance of bacterial endocarditis and one a patient with pyelitis, showed an increase up to 250 units, a level which most observers consider to be highly significant. Since it has been remarked repeatedly that the antistreptolysin titer of the serum in infections due to hemolytic streptococci does not increase until the disease is well advanced or until convalescence has been well established (3, 4, 5), it is important to know the day of disease on which the tests were made. The serum from three of the patients with typhoid fever was tested between the third and sixth week of the disease, and from three patients between the seventh and eleventh week of the disease. In one of these patients the serum was tested at weekly intervals for three weeks. The titer remained constantly at 50 units. In one, the serum was tested weekly for four weeks. The titer in the eighth week was 100 units; in the ninth, tenth and eleventh weeks it remained at a constant titer of 111.1 units. The sera from the cases of malaria, of meningococcal meningitis and of gonococcal arthritis were titrated only during the acute phase of the disease. The sera from the miscellaneous cases were tested at various stages of their diseases, and the sera from the cases of bacterial endocarditis weeks and months after the onset of their illness.

Especial interest attaches to the examination of the patients with lobar pneumonia, for Todd (17) has reported the fact that antistreptolysin serum of high titer will neutralize the oxygen labile hemolysin produced by pneumococci and by *B. tetanus*. The reaction was found to occur, however, only with "hyperimmune serum." This partial overlapping was not noticed when the serum from patients suffering from hemolytic streptococcal infections, which is of low titer compared with the serum from immunized animals, was studied. The serum from nine patients convalescing from lobar pneumonia due to pneumococcus Type II were tested for antistreptolysin. The serum from only one patient gave an abnormally high figure of 250 units.

The sera from 24 cases of lobar pneumonia, which are listed in Table III, were collected from

the third to the fifth week of the disease. The sera from 20 patients gave titers on repeated examination that were quite constant and always within normal limits. The sera from 4 patients gave titers that were equally constant but slightly above normal. The titrations were made in the second, third and fourth week of the disease. The titers varied from 125 units to 166.7 units. The pneumonia in three of these patients was due to pneumococcus Group IV and in one to pneumococcus Type I. The blood cultures were negative in all four cases and all recovered. Of the remaining 20 cases, 5 gave positive blood cultures; two of these died, three recovered. Three patients infected with pneumococcus Type I received concentrated antipneumococcal serum and recovered. Of the 24 cases, 14 were infections due to pneumococcus Type IV; 7 were due to pneumococcus Type I; one to pneumococcus Type II; one to pneumococcus Type III, and in one the organism was not identified.

The proportion of slightly elevated titers was, therefore, not quite as large in these patients suffering from pneumonia as it was in the meningococcal and gonococcal infections.

#### SUMMARY

In reviewing the results obtained from titrations for antistreptolysin in the sera from normal individuals, and from patients suffering from a variety of chronic diseases and acute infections not caused by hemolytic streptococci, it is seen that they accord in general with those previously published. It appears justifiable to conclude, as Todd did originally, that the upper limit of antistreptolysin in the serum from the normal adult is 100 units. In the majority of cases (about 75 per cent) the titer in our series lay between 25 and 50 units. The determination of the lower limit of the antistreptolysin in the normal adult is also important, for Lippard and Johnson (9) found this to be unusually low in infants under 17 months of age. In 55 per cent of these patients the titer was only 2 units. The lower level in our normal adults was 12.5 units. It is also to be observed that the titer varies very little from month to month in a given individual. The titer appeared to be unaffected by severe colds and minor infections not due to hemolytic streptococci,



but even a comparatively insignificant infection caused by hemolytic streptococci may result, as it did in Number 4, in a significant rise in the antistreptolysin titer within 18 days of the infection. Since such rises, as has been repeatedly shown, may persist for weeks or months, it would not be surprising to find a moderately elevated antistreptolysin titer in the serum of individuals who were apparently healthy, who showed no evidence of infection by hemolytic streptococci and who were actually unaware of having had such infections.

In view of this possibility it is noteworthy that the antistreptolysin content of the sera from patients suffering from chronic diseases and from a miscellany of infections should approach so closely the normal figure. In the examination of 155 specimens of serum from the 84 patients with chronic disease only a total of 16 samples showed titers above 100 units and none of these was above 166.7 units. The total number of patients furnishing serum showing titers above 100 units was 14, or 16.6 per cent of the total.

Of the 155 samples of serum from the 84 patients, 139 gave titers within the average limits of normal.

In the acute infections the number of slightly elevated titers was proportionately greater, for of the 113 samples of serum from 66 patients, 22 samples from 15 patients gave antistreptolysin titers somewhat above the figure for normal adults. Titers above 100 units occurred in 22.7 per cent of the 66 patients.

It would be impossible to determine, at the present time, whether the infecting organism, such as the meningococcus or pneumococcus, was in any way related to these abnormal rises, or whether an infection by hemolytic streptococci contracted previous to, or in association with the present infection was responsible for the abnormal titer. Owing to the prevalence of hemolytic streptococci it would be impossible to exclude the latter possibility.

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TABLE I

*Antistreptolysin titers (units per cc.) of the blood serum of 19 cases of erysipelas*

Time of determination	Patient number																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16*	17	18	19
day																			
1																			
2				25	50.0						125				125	125*		125	166.7
3													333.3			125	25		
4														33.3		125			
5	50.0							50.0											
6																			
7															125				
8									250										166.7
9	50.0		250																
10			250										333.3						
11			250											50.0					
12																			
13																			
14						250	250												166.7
week						250												125	
3								125		250			333.3						
4						250													
month																			
2	166.7											50.0							
												50.0							
												50.0							
												50.0							
3												50.0						125	
												50.0							

\* One month after infection of throat due to *B. hemolytic streptococcus*, and 1 week after acute pharyngitis due to *B. hemolytic streptococcus*.

TABLE II

*Antistreptolysin titers (units per cc.) of the blood serum from 18 cases of scarlatina*

Time of determination	Patient number																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16†	17	18
day																		
1					*					*	25		*	50.0			*	*
2	*	100				*		125						*			125	
3	50.0																	
4																		25
5																		
6					25											50.0		
7											50.0							
8							25	125						50.0				
9				25														
10																		
11						25												
12								142.9		50.0								50
13														100		111.1		
14																		
week																		
3		125	250		33.3				166.7	100		100			166.7			50
		166.7				33.3			166.7				250			166.7		50
4																		
month																		
2	166.7																	

\* Scarlatinal antitoxin.

† Puerperal infection and scarlatina.

that during the first few weeks of the disease there was usually a rapid increase in the antistreptolysin content of the serum, but the numbers of observations are too small to enable one to demonstrate that this actually occurred in each individual. In Case 8 the antistreptolysin titer rose from 50 units in the first week to 125 units in the third week. In Cases 6, 13, 18 and 19 the titers remained unchanged throughout the course of the disease, and in Case 13 remained constantly at an elevated titer of 333 units for 3 weeks.

In scarlet fever, though our observations are meager, they nevertheless show that there is the same tendency towards an increase in antistreptolysin titer of the serum in the third week of the disease (Table II). The titers are not very high, and an increase above 100 units was only noticed in 8 of the 18 cases, and above 200 units in 2 cases.

TABLE III

*Antistreptolysin titers (units per cc.) of the blood serum from 14 cases of acute tonsillitis*

Time of determination	Patient number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
day														
1														
2		25			100									
3												50.0	50.0	
4														
5		100												
6														
7								50.0		25				
8				125									50.0	166.7
9							16.7							
10												50.0		
11														
12														333.3
13														
14														
week														
3						125	25				250	125		
4	50.0											200		
month												200		
2			25									250		
3												333		
												200		

In tonsillitis these elevations were even less common than they were in erysipelas and scarlatina (Table III). There is, however, again a tendency for the titer to rise during the latter part of the disease or in convalescence. Five of the fourteen cases gave titers above 100 units in the second or third week and three of these gave titers of 200 or above. Case 12 demonstrates very well the rise of the antistreptolysin titer in

the third week of the disease and the persistence of high titers (after recovery) for three months.

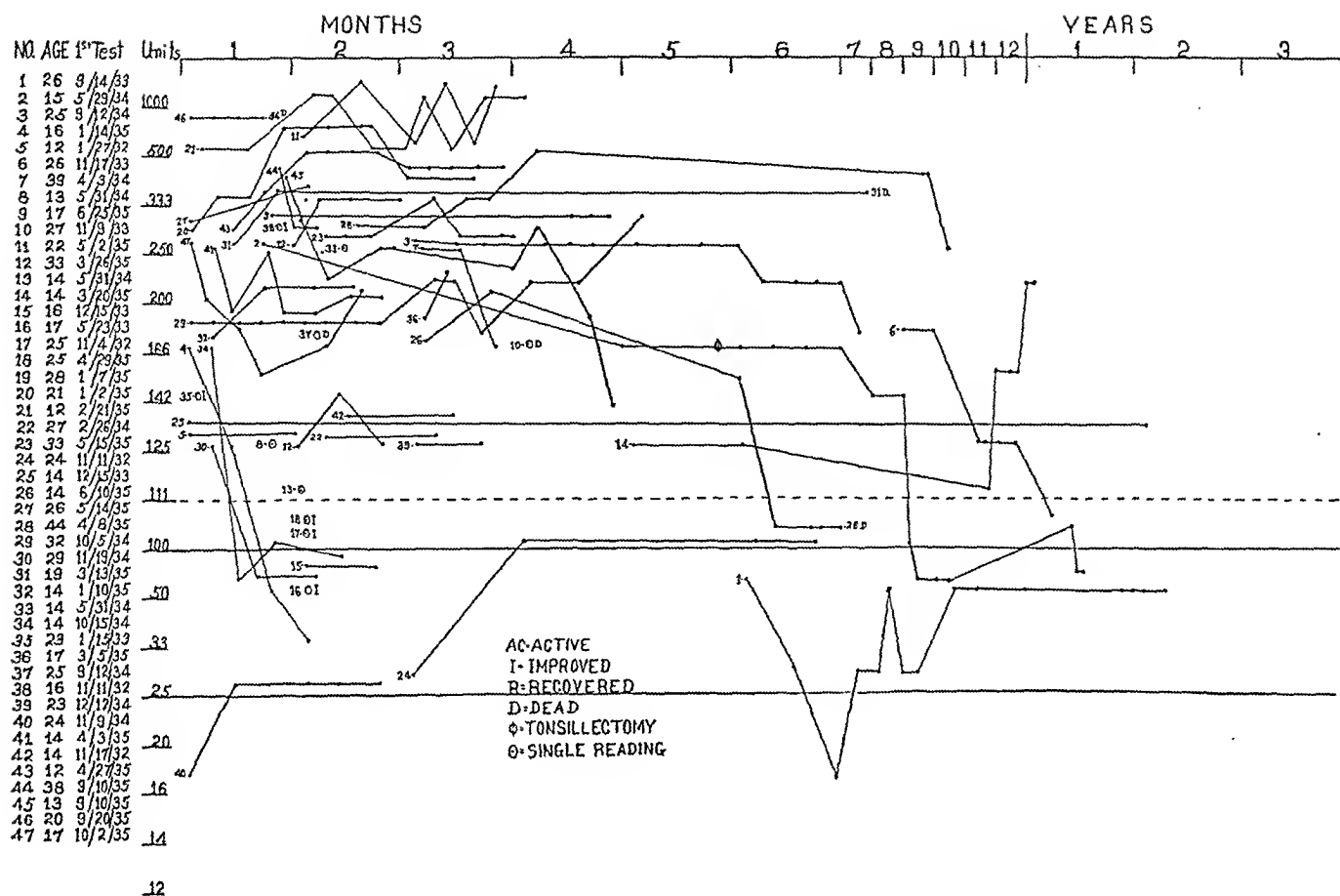
In the group of miscellaneous infections due to hemolytic streptococci there are only 7 cases (Table IV). Three of these showed an increased

TABLE IV

*Antistreptolysin titers (units per cc.) of the blood sera from 7 cases of miscellaneous infections due to hemolytic streptococci*

Time of determination	Patient number						
	1	2	3	4	5	6	7
day							
1							33.3
2							
3							
4		25					
5				333.3			
6				500			
7							
week							
2				1250	50		
3				1429		50	125
4				500			
month							
1 to 2				555		50	
						33.3	125
						33.3	
2 to 3						25	
3 to 4	250		25				

antistreptolysin titer above 100 units. The number of cases is so small that they are important only on the following account. Case 4 was a boy, age 13, who developed a severe infection perforating the skull and involving the brain. From this pus and from secondary abscesses Drs. Long and Bliss cultivated minute hemolytic streptococci in pure culture. Minute hemolytic streptococci were also cultivated from the antrum in Case 6. In view of the fact that at the present time there is no information as to whether minute hemolytic streptococci are capable of inciting antistreptolysins for the oxygen labile streptolysin formed during the growth of hemolytic streptococci Group A of Lancefield, these observations are significant. Todd (3) has recently published a comparative study of the streptolysins and antistreptolysins produced by strains belonging to the different Lancefield groups (3a). It was found that the antistreptolysin produced by a strain from Lancefield Group A (human) did not neutralize hemolysin elaborated by strains from



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FIG. 1. CURVES OF ANTISTREPTOLYSIN TITER OF THE BLOOD SERUM FROM 47 CASES OF ACUTE RHEUMATIC FEVER.

ready been published on this subject, but they are of value since they furnish data derived from patients with rheumatic fever in Baltimore and may therefore be used for comparative purposes in a study of the antistreptolysin content of the serum from cases of hemorrhagic nephritis, occurring in the same region.<sup>2</sup>

<sup>2</sup> It has become obvious that these control studies of the antistreptolysin content of the serum from normal individuals, from patients suffering from chronic diseases and from infections other than those due to hemolytic streptococci, as well as from infections due to hemolytic streptococcus and from rheumatic fever are important, for Coburn and Pauli (10) have recently shown that the antistreptolysin content of the serum in healthy individuals may vary to a certain extent according to the latitude in which these individuals live. They have collected statistics to show that below latitude 35° the median value for healthy individuals was 71 units and that serum from 78 per cent of the individuals showed a titer below 100 units. Above latitude 35° the median titer of antistreptolysin of serum from 231 individuals was 100 units and the serum from only 58 per cent of the individuals showed an antistreptolysin titer of less than 100 units. It will be observed that throughout our observations the antistreptolysin titers are generally somewhat lower than those reported from Boston and New York both for healthy individuals and for individuals affected by hemolytic streptococci.

As our experience with acute nephritis has increased we have, from time to time, over the period in which we have been studying it, altered our views with regard to many phases of the disease and modified somewhat our conception of the process. It now seems probable that the disease which is designated as diffuse glomerular nephritis may assume two more or less distinct forms. These two forms have been very well differentiated by Winkenwerder, McLeod and Baker in a survey of our cases (5).

The first is the more common. This form follows in its general symptomatology and course the descriptions usually given of the disease. The onset follows within 5 to 21 days a more or less severe infection of the tonsils, respiratory tract or more rarely of the skin. It is the form that complicates scarlet fever. The infection preceding it is in the vast majority of cases due to hemolytic streptococci. The onset of symptoms is usually abrupt. In the severe cases there is fever, nausea, vomiting, pain in the lumbar region, suppression of urine or anuria. A peculiar form of anasarca develops rapidly. It produces a firm gelatinous swelling of the tissue, with very little

pitting. There is a precipitous rise in blood pressure, occasionally acute dilatation of the heart and sometimes dyspnea. Costovertebral tenderness is frequent. There is albuminuria, sometimes of only moderate degree, but the urine contains numerous red blood cells, casts of many varieties and frequently many leukocytes. Rarely hemorrhages and exudates are seen in the retina. In the severest cases, there is reduction of phthalein excretion, elevation of nonprotein nitrogen of the blood and a low urea or xylose clearance.

Milder forms are quite common. In these there may be no fever, only slight facial edema, only transient hypertension, and no alteration in the renal function except for the inability to concentrate the urine normally. The hematuria, albuminuria and cylindruria are distinctive.

The particular features that distinguish this variety from the second form are the preceding acute infection always accompanied by a more or less severe constitutional reaction, the sudden onset, the time of which is often unmistakable, and the rapidity with which many cases improve. In rare instances the patient dies either from the original infection, an associated infection such as pneumonia, or in acute uremia. Much more often the improvement from the acute stage even in severe cases is comparatively rapid. The patient may later pass through various phases of the disease and experience exacerbations with recurring streptococcal infections, or in unfavorable cases pass into a nephrotic stage much like that observed in the second form. Eventually, however, recovery or complete quiescence takes place in a fair majority of patients (85 per cent of 61 cases of this type). Once complete recovery has been established, recurrences and exacerbations have not been observed, even though the patient might suffer at one time or another from an acute infection due to hemolytic streptococci.

The second form (designated Type B) is distinctly less common. Two distinguishing features are; first, that the disease is not usually preceded by any infection of which the patient is aware, and secondly, that the onset is so insidious that the exact time of its appearance can not often be accurately ascertained. In the majority of instances the patient first notices swelling of the feet, which gradually involves the entire body.

In other instances albumin is found accidentally in the urine, and edema appears later. Usually, there are no other initial symptoms. The blood pressure is normal or only moderately elevated, the retinæ show edema, but no exudates or hemorrhages. Albuminuria is marked, there is hematuria of varying degree and cylindruria. The renal function, otherwise, is rarely affected. Careful examination usually reveals some chronic or indolent infection. This usually affects the accessory facial sinuses, the tonsils or the lower respiratory tract. The infection is often, but not always associated with hemolytic streptococci, sometimes of the alpha type. At times only the minute form of hemolytic streptococci, described by Long and Bliss (5a), has been obtained in culture. The course is often punctuated by acute exacerbations with increased blood pressure, gross hematuria and increase in albuminuria. Though the persistent dropsy is one of the characteristics of this type of the disease, edema may almost disappear, spontaneously, following such an acute exacerbation. Edema usually persists for months and may be present off and on for years. The patient may die in the subacute nephrotic stage a few months or a year or two after the onset, or may progress to a quiescent stage, with little or no edema, and only moderate hypertension, but with marked albuminuria and constant microscopic hematuria that varies in degree from time to time. Under these circumstances, the disease often terminates with hypertension, and the patient dies in uremia with some edema. In 25 cases of this type there were no complete recoveries, for the disease was fatal or resulted in a slowly progressive chronic and incurable nephritis in all.

It has seemed desirable, therefore, to consider the antibody response to streptolysin as it was observed in three separate groups of cases of nephritis. Types A and B include all the instances of hemorrhagic nephritis of which there were 97. Type C does not include any instances of hemorrhagic nephritis.

There were 78 cases of acute active hemorrhagic nephritis belonging to the form first described (Type A). Thirty-six of these patients have recovered, 17 of them having been followed

TABLE VIII

*Highest antistreptolysin titers of serum from 57 cases of hemorrhagic nephritis (Type A), 19 cases of hemorrhagic nephritis (Type B) and 18 cases in the miscellaneous group (Type C)*

Diagnosis	Antistreptolysin titers ( <i>units per cc.</i> )																	Total number of cases	Titers above 100 units		Titers above 200 units	
	10	12.5	14.2	16.7	20	25	33.3	50	100	111.1	125	142.9	166.7	200	250	333.3	500		Number	Per cent	Number	Per cent
Acute hemorrhagic nephritis (Type A)																						
Acute stage.....				1		3	1	4	1		3	1	7	3	7	3	2	36	26	72.2	15	41.6
Quiescent and chronic.....				1		5	2	9	5		4		4		1			31	9	29	1	3.2
After recovery from nephritis...				3		14	2	8	3		1	1	1	1		1	1	36	6	16.6	3	8.3
Hemorrhagic nephritis, latent onset (Type B)																						
Latent onset, active stage.....				2	1	1	1	7	1	2	3		1					19	6	32.1	0	
Miscellaneous group (Type C)																						
Miscellaneous, highest titers...	1		1	1	2	2	1	7					3					18	3	16.6	0	
Miscellaneous, lowest titers....	4	3		1		2	1	4	1				2					18	2	11.1	0	

from the acute stage of the disease. The streptolysin content of the serum from the remaining 19 cases was determined only after recovery. There were 40 patients who are now in a completely quiescent stage of the disease, or in whom the disease has progressed to a chronic stage or has been fatal. Four of the deaths occurred during the acute stage of the disease, 4 during the chronic stage. Nineteen of these 40 patients have been examined repeatedly from the acute stage of the disease throughout their illness. Two patients, in addition to those already cited, have been examined and are still in the acute stage of the disease.

Table VIII gives a résumé of the principal data bearing directly on the problem under investigation in all patients grouped in Type A. The infections preceding the onset of the acute nephritis in the 78 patients designated as Type A were: scarlatina in 6 cases, erysipelas in 2 cases, and a severe tonsillitis, sinusitis, bronchopneumonia or infection of the skin in 59 cases. In three cases the acute nephritis, which was quite severe in one instance, developed during an acute attack of rheumatic fever with rheumatic heart disease. A severe acute infection, therefore, was known to precede immediately the onset of the

symptoms of acute nephritis in 70 of the 78 cases. Excluding the instances of scarlatina, erysipelas and rheumatic fever, cultures were obtained either from the infected tissue or from the seat of the infection at different periods during the course of the nephritis in 66 of the remaining 67 cases. Either hemolytic streptococci of beta type, or the minute type, or of both types were obtained in culture from 58 of these 66 cases. It is important to note, therefore, that more or less severe acute infections due to hemolytic streptococci were known to initiate the symptoms of acute nephritis in the vast majority of the cases grouped as Type A.

There were 19 cases of hemorrhagic nephritis with latent onset, described as the second type "B." Four of these have died, the others have been examined repeatedly during the active progressive stage of the disease.

In contrast to the patients belonging to the form of acute nephritis termed Type A, the patients in Type B rarely gave a history of an acute infection, and when upon examination an infection was discovered, it was found usually to be chronic in nature. Chronic infections, often of the facial sinuses, were actually detected in 11 of

the 19 cases. In several instances these infections were unsuspected by the patient. These chronic infections were usually associated with hemolytic streptococci, for in 15 of the 19 cases hemolytic streptococci of one or another type were cultured in significant numbers and often on several occasions from the nasopharynx, tonsils, or from the discharge from the sinuses.

In Type C have been placed a miscellaneous collection of cases of renal disease, including pyelonephritis, bichloride of mercury nephrosis, amyloid nephrosis, and indeterminate forms of Bright's disease seen in the chronic edematous stage. There were 18 of these cases, many of which were observed for several months.

Table VIII presents the highest antistreptolysin titers of the serum from the nephritic patients belonging to all three types. It will be seen immediately that the greatest number of high titers was obtained with the serum from patients studied during the acute phase of the disease (Type A). All patients in Type A examined during this stage of hemorrhagic nephritis, including those that died during the acute phase, those that progressed to a chronic course, and those that recovered, have been placed in this section. There were 36 patients, 26 of whom, or 72.2 per cent, gave titers above 100 units, and 15 of whom, or 41.6 per cent, gave titers above 200 units.<sup>3</sup> Of the total number, 17 recovered, 13 progressed either to a quiescent or chronic stage, 4 died during the acute attack, and 2 are at present still in the acute phase of hemorrhagic nephritis.

In the chronic or quiescent stage of the disease (Type A) high titers were occasionally met with in some patients, but they were much less common than during the acute stage. Only 9 of the 31 cases gave titers above 100 units, and only 1 patient gave a titer above 200 units. In the healed stage of the disease (Type A) after complete recovery, high titers were still less common, for only 6 of the 36 cases gave titers above 100 units. Five of the six cases that gave titers above 100 units were examined within one year after the onset of the nephritis.

<sup>3</sup> Since the compilation of this data, 8 additional cases of hemorrhagic nephritis Type A, in the acute stage, have been examined. In 4, the antistreptolysin titers of the serum were between 111 and 250 units; in 2, it was 1250 units; in 1, 1666 units; and in 1, 2,000 units.

The situation in this group of patients (Type A) is somewhat analogous to that noticed in rheumatic fever. Though the increase in antistreptolysin in the serum from patients with acute hemorrhagic nephritis is not quite so common and does not often reach quite so high a titer as it does in acute rheumatic fever, the results approximate more nearly rheumatic fever than any of the outspoken instances of infections due to hemolytic streptococci that we have studied. In addition, the same relationship holds between the two diseases in the acute and healed stages.

When the antistreptolysin response in the active stage of the disease in patients belonging to Type B, with latent onset, is compared with that in the acute hemorrhagic form, Type A, the difference is very striking. The determinations were frequently made at approximately the same time after onset of the disease in these two groups. In the patients with latent onset the highest titers rarely exceeded 100 units and in no case were they above 200 units.

Finally the 18 patients in the miscellaneous group of nephropathies, termed for convenience Type C, present interesting features. Repeated determinations of the antistreptolysin titers were made on many of these patients over several months, and consequently it scarcely seems possible that the unusual results are due to chance findings. Both the highest and the lowest titers are recorded. The characteristic feature is the very low titer of the serum obtained in several patients. This is frequently comparable to the low titers obtained by Lippard and Johnson (6) in infants under 18 months of age. All of these patients with very low titers were edematous. In some patients the antistreptolysin persisted at abnormally low titers for months. The serum from one patient gave titers that varied between 5.5 units and 10 units in 14 tests over a period of four months. A second patient gave titers on 9 occasions varying from 8 to 10 units over a period of 6 months, and a third patient gave titers on 7 occasions varying from 10 to 12.5 units over a period of 6 months. No explanation can be offered for these abnormally low titers of antistreptolysin.

Since it was desirable to obtain information concerning any possible relationship that might obtain between the variations in antistreptolysin



content of the serum and the progress of hemorrhagic nephritis, the antistreptolysin curves have been charted week by week and month by month in 17 of the 36 cases who recovered (Figure 2), and in 19 of the 40 patients who are now in the quiescent stage, who have progressed to a chronic state or who have died (Figure 4).

rhagic nephritis as they were in acute rheumatic fever, and that a larger proportion did not rise above the limits of normal at any time during the course of the disease.

No constant relationship could be found between the severity of the attack of acute nephritis and the height of the antistreptolysin titer, for

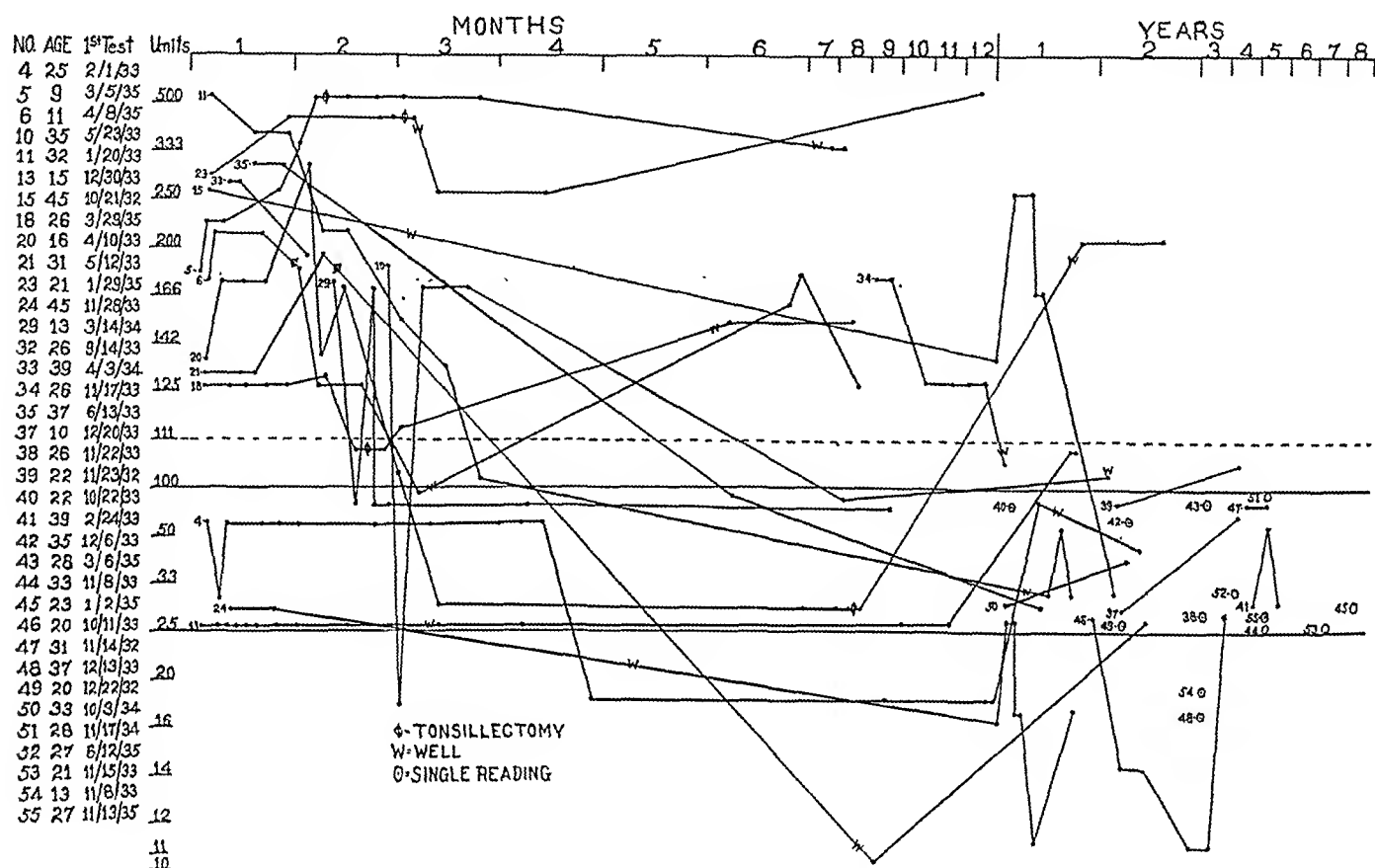


FIG. 2. ANTISTREPTOLYSIN CONTENT OF SERUM FROM 36 PATIENTS WITH HEMORRHAGIC NEPHRITIS TYPE A WHO HAVE RECOVERED.

The curves for the patients who have recovered have been charted in Figure 2. In general the trend of the curves simulated that obtained in acute rheumatic fever, inasmuch as the antistreptolysin titers in the majority of the patients were high in the first weeks or even months of the disease. As convalescence was established and as recovery took place, the curves tended in several cases to decline to the normal levels. One to five years after complete recovery was established, the titers from all but two of 29 cases were within normal limits, and in 24 cases lay between 16.7 and 50 units. Though the general appearance of the curves resembles that in acute rheumatic fever, it was found, as the chart shows, that the original titers were not so high in acute hemor-

some patients, suffering from much the same form of infection preceding the attack of nephritis, showed totally dissimilar curves, with high titers in one and persistently low titers in another (Figure 2, Number 4, and Figure 4, Number 19). The rapidity with which the titers fell to low levels did not seem to be regularly allied to the form of infection caused by hemolytic streptococci, to the severity of the nephritis, or to the rapidity with which the patients recovered from the nephritis (Figure 3). After comparatively mild infections, followed by attacks of nephritis of only moderate severity with rapid recovery, the antistreptolysin titer remained high in some patients for months, whereas in others, having severe infections followed by alarmingly severe attacks of

nephritis, the titers fell rapidly with recovery (Figure 2, Number 5; Figure 3, Number 11). Whenever the titer was high during the acute phase of nephritis, it remained elevated in most cases beyond the time at which all evidences of nephritis had entirely disappeared (Figure 2, Number 5).

Another interesting irregularity was the sudden secondary rises in titers after the patient had recovered and after the antistreptolysin content of the serum had dropped to a normal figure. This

streptococci in the pharynx of these patients might account for such irregularities. Examination of the bacteriological flora of the throat from the patients who have recovered have been made in many instances by Dr. Long, within a few months or weeks of the time that the titers of the serum were determined. Seventeen of the cases have given at least one positive culture; 16 of the cases did not at any time show hemolytic streptococci, and in three cases cultures were not obtained. Since 12 of the cases with low titers

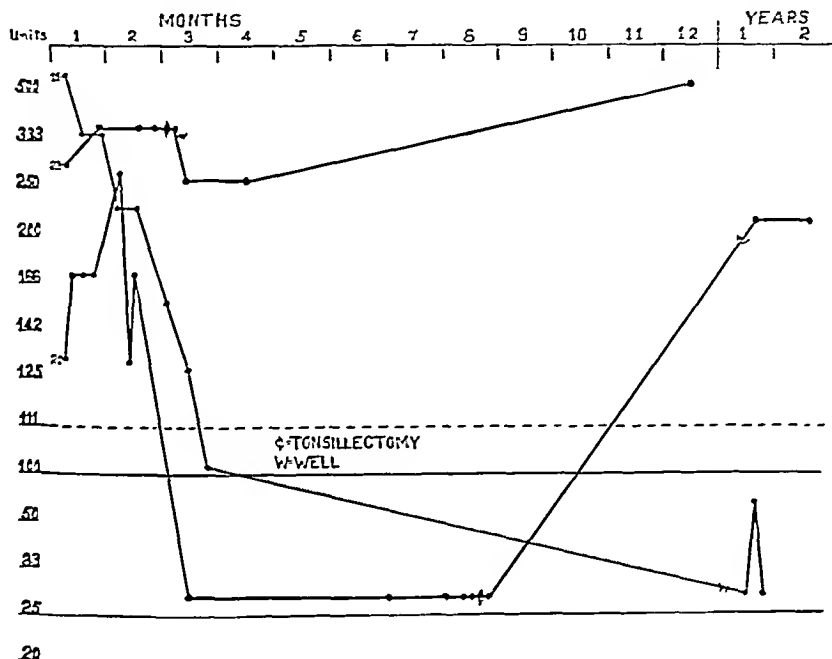


FIG. 3. ANTISTREPTOLYSIN CONTENT OF SERUM FROM 3 CASES OF ACUTE HEMORRHAGIC NEPHRITIS TYPE A THAT HAVE RECOVERED.

occurred in seven patients. In one (Figure 2, Number 6) the secondary rise could be explained by the fact that the patient contracted an attack of impetigo due to hemolytic streptococci. This infection was not accompanied or followed by signs of exacerbation of hemorrhagic nephritis. One of the other four patients had rheumatic fever, but the rise of titer in the remaining 5 cases was not connected, as far as could be discovered, with any infection due to hemolytic streptococci (Figure 3, Number 20). In one patient (Figure 2, Number 15) the secondary rise was of comparatively short duration. It is, however, possible that the mere presence of hemolytic

gave, on at least one occasion, a positive culture for *B. hemolytic streptococci*, it hardly seems possible that the temporary carrier state could account for high antistreptolysin titers. All of the patients with high titers after recovery showed hemolytic streptococci in the throat culture on one or more occasions, but in three of the five cases in which an adequate explanation was not found, the examinations and titers were made within 4 to 7 months of the acute attack of nephritis. In one instance, Figure 3, Number 20, the high titer was obtained 2 years after the attack.

The antistreptolysin curves for 19 patients in whom the acute hemorrhagic nephritis (Type A)

progressed to a quiescent stage, became chronic or caused death, are charted in Figure 4. They present a somewhat different appearance from those in Figure 2, for the curves usually remain persistently higher or are broken by sudden eleva-

patients the titer was high during the first few weeks of the disease (during first month in 21 and during first two months in 31 of 35 cases), though in a few the titer never rose above the normal figure. There was a tendency for the

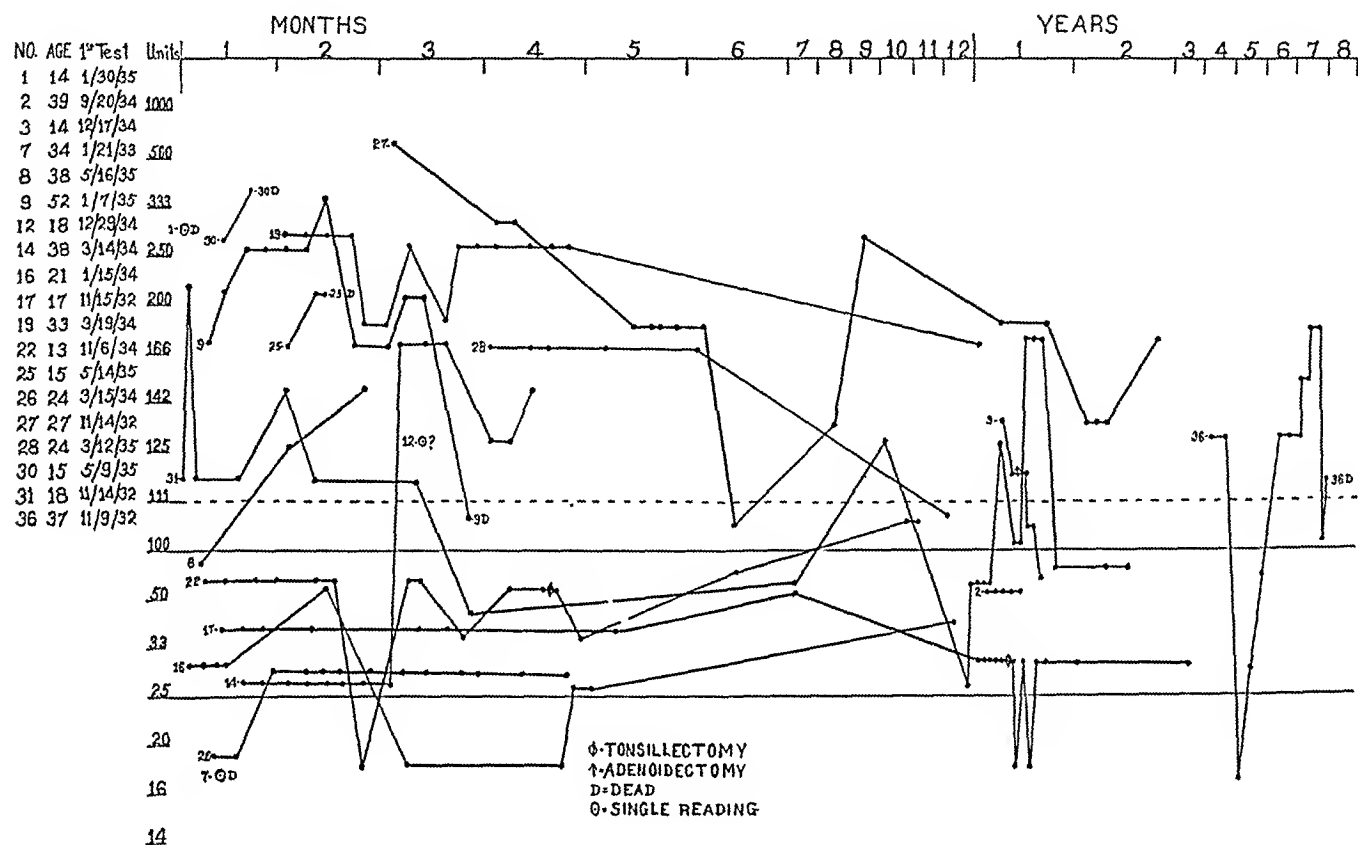


FIG. 4. ANTISTREPTOLYSIN CONTENT OF SERUM FROM 19 PATIENTS WITH HEMORRHAGIC NEPHRITIS TYPE A WHO HAVE PROGRESSED TO LATENT OR CHRONIC STAGE OR WHO HAVE DIED.

tions which are coincident with remitting acute infections accompanied by exacerbations of the nephritis. This is especially noticeable in Cases 19, 27 and 31 (Figure 5). It is also to be observed that high titers persist in some cases for many months or years (Figure 5, Number 27) and were found in one case (Figure 4, Number 36) from 4 to 7 years after the primary attack of nephritis. In a few cases, notably Numbers 17 and 26 (Figure 4), the titers never rose above normal. One of these patients has been followed for 3 years.

From a study of the curves of the antistreptolysin titers in these 36 patients, 17 of whom recovered (Figure 2), 19 of whom are now in quiescent stage, have progressed to a chronic stage or are dead (Figure 4), it may be said that no very constant relationship was found to exist between the form of the disease and the curve of the antistreptolysin titer. In the majority of all

antistreptolysin titer to fall as the patient recovered, but high titers were obtained weeks or months after complete recovery had been established, and occasionally there was a secondary rise, months or even a year or two following complete recovery and after the titer had previously reached normal.

While the disease was still quiescent or when it progressed to a chronic stage, the antistreptolysin titer remained at a high level in a fair proportion of instances, and in some persisted at these levels for years.

It is important to bear in mind that in the vast majority of these patients comparatively severe infections by hemolytic streptococci preceded the onset of the nephritis by an interval of a few days to a few weeks, and that in many of the chronic cases evidence of infection persisted throughout the course of the disease.

The 19 patients of Type B with latent onset

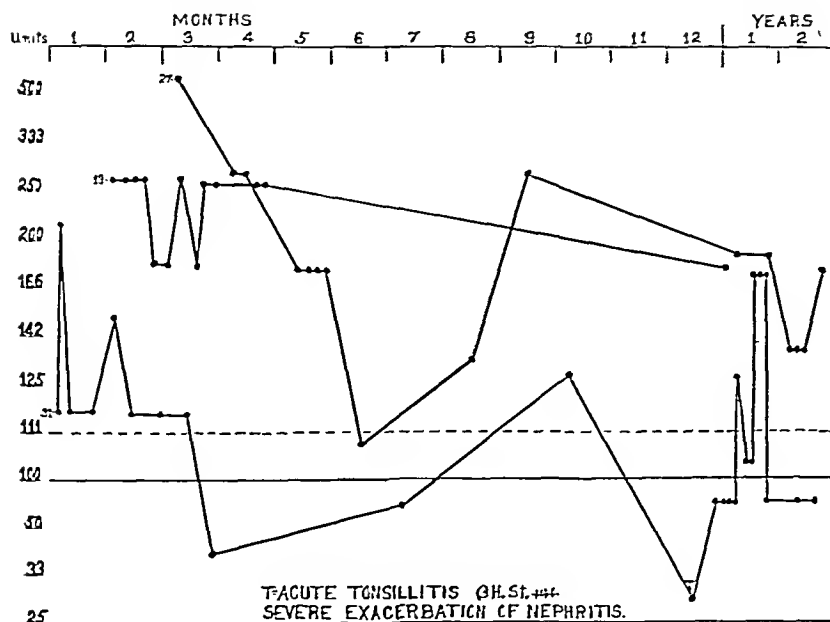


FIG. 5. ANTISTREPTOLYSIN CONTENT OF SERUM FROM 3 CASES OF ACUTE HEMORRHAGIC NEPHRITIS TYPE A IN QUIESCENT OR CHRONIC STAGE.

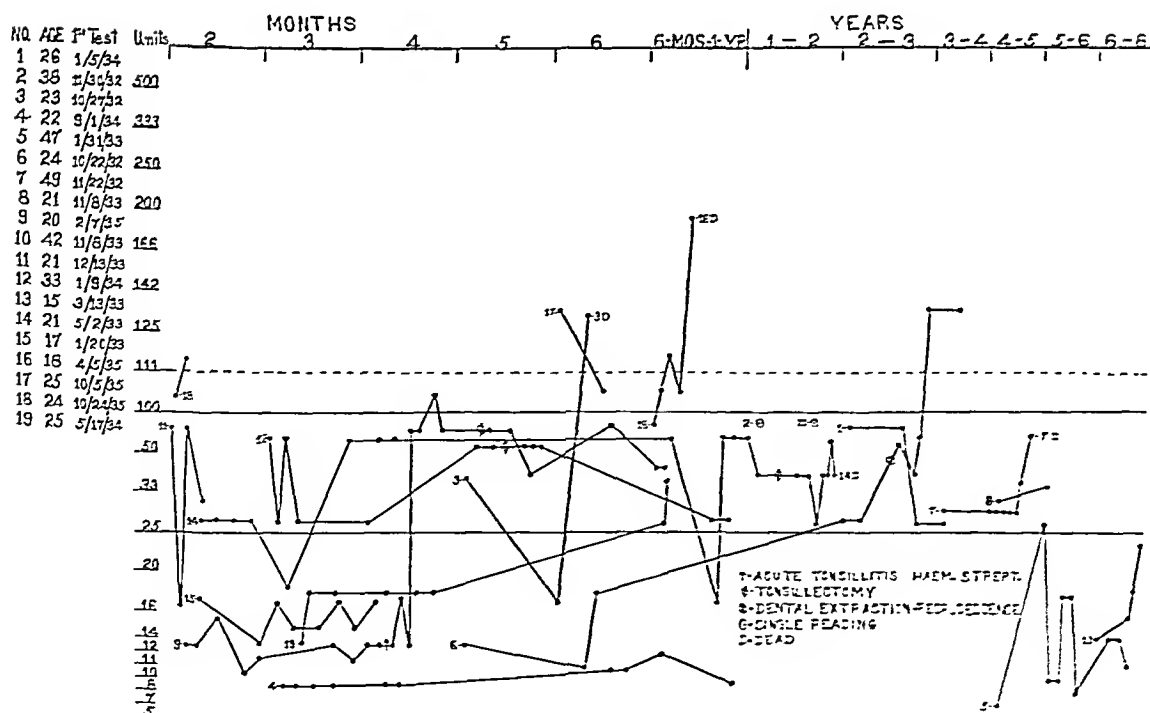


FIG. 6. ANTISTREPTOLYSIN CONTENT OF SERUM FROM 19 CASES OF HEMORRHAGIC NEPHRITIS TYPE B.

present an interesting problem. Antistreptolysin titers were determined in at least 8 of them during the first three months of the disease, or as near this time as could be determined from the histories (Figure 6). The characteristic of these cases (Type B) was the persistently low level of the antistreptolysin titer. In only 5 out of the 19 cases did the titer rise at any time above the normal level, and in 2 this occurred just before death. It will also be noted that these low levels persisted for months and years. Two cases are of particular interest, Numbers 9 and 6 (Figure 6). Patient Number 9 experienced, during the fourth month of her disease, a severe acute attack of tonsillitis due to *B. hemolytic streptococci*. This was accompanied by gross hematuria. Immediately after this acute attack, the antistreptolysin titer rose from 12.5 units to 16.7 units, and during the next two weeks rose abruptly to 100 units. During the following two months the titer varied from 50 units to 33.3 units, but at no time, even after the acute infection, did the titer rise above normal. Patient Number 6 had shown variations in titer between 10 and 25 units for about 2 years when suddenly after an infection about a tooth, which later was extracted, the titer rose from 50 to 125 units, where it has remained for 8 months.

Another interesting feature is the very low titer which at times these patients may show. Titers between 7 and 10 units were obtained on one or several occasions with the blood serum from 4 of these patients (4, 5, 6, 9).

The explanation for the persistently low titers in the patients of this type is not clear. Practically all of them have had or now have more or less marked edema though, in several, during the latter stages of the disease this has been confined to slight pitting over the shins. In all, the plasma proteins have been reduced below the normal level. Though many of them have infections, often caused by hemolytic streptococci, the local reaction to the infection is sluggish, and, as has been pointed out by Winkenwerder, McLeod and Baker (5), the onset of the disease, being insidious, is quite different from that observed in the patients grouped in Type A. It is possible that the reactive processes of the body are partially suppressed in this group. This might result in

an ineffective reaction against the infection as well as against the lesions in the kidney; and the low titers of antistreptolysin may be actually another indication of the lack of response of the protective mechanism. Another explanation might be found in the possibility that the infection and the accompanying nephritis is primarily associated with an organism other than the hemolytic streptococcus. Rake and Blackman (7) have recently recalled attention to the fact that acute hemorrhagic nephritis may occur as a result of infections by pneumococci, and Seegal (8) supports this view. Blackman (9) has been able to produce experimentally in rabbits through the repeated intravenous injection of pneumococcus toxin a form of nephritis accompanied by edema.

It might be argued, therefore, that the hemolytic streptococci occur in these patients accidentally, or as an inactive associated organism, and thus cannot be regarded as the immediate cause of the infection. The fact remains, however, that in at least one patient a severe tonsillitis due to hemolytic streptococci was not followed by a rise in titer above normal.

Discussion of the conditions in the third group is not necessary, since there was no indication that the various forms of disease of the kidney were in any way connected with infections due to hemolytic streptococci.

#### DISCUSSION

The observations that we have made accord with those of others, and add further evidence to show that infections by hemolytic streptococci are sometimes accompanied, but are more often followed, by a significant rise in the antistreptolysin content of the blood serum. The rise does not usually occur until one to three weeks after the onset of the infection. It reaches its highest titer promptly, and, then, after a variable length of time, falls sometimes rapidly but frequently slowly to the normal level. The period during which the antistreptolysin titer persists at an abnormally high level is extremely variable. The titer does not always fall immediately with recovery from the disease, for high titers may be encountered for weeks or months after the patient is entirely well. This may occur following mild as well as severe infections. On this account, if

for no other reason, the test cannot be employed for diagnostic purposes, unless the actual change from a normal titer to a high titer occurs under observation.

The height to which the antistreptolysin titer rises does not seem regularly to be dependent upon the severity of the infection. Indeed as was first noted by Todd (1), fatal infections by hemolytic streptococci may not be accompanied by any increase in the antistreptolysin content of the blood. Coburn and Pauli (2) have, however, presented evidence to show that the height of the antistreptolysin titer parallels to a certain extent the severity and duration of acute attacks of rheumatic fever, but this has not been the usual experience with streptococcal infections in general, nor has it occurred in our small series of cases.

There appears, also, to be some variation in the production of abnormal amounts of antistreptolysin in different forms of streptococcal infection. In our series, increase in antistreptolysin frequently did not occur after acute tonsillitis and pharyngitis, and in the patients in which the antistreptolysin did increase it rarely reached a high titer. Coburn and Pauli (2) have called attention to this same occurrence in their series. An increased antistreptolysin was more common in scarlatina and high titers more frequent, while antistreptolysin was regularly increased in erysipelas, and high titers were the rule. Under these circumstances it appears somewhat peculiar that in rheumatic fever and hemorrhagic nephritis, both of which are so commonly preceded by acute streptococcal tonsillitis and pharyngitis, the antistreptolysin should be so regularly elevated to a high titer during the acute stage of the disease.

One might advance this as an argument to uphold the view that both diseases are caused by hemolytic streptococci, but it is obvious, from the previous experiences, that persistently high titers of antistreptolysin may be obtained for weeks following all varieties of streptococcal infection. This raises a valid objection to using this test as an indication that any complication of a streptococcal infection is in itself due to hemolytic streptococci. The observations can undoubtedly be interpreted, however, as meaning that both diseases are preceded with remarkable constancy by infections due to hemolytic streptococci, but

scarcely warrant any further conclusion in regard to the etiology of acute hemorrhagic nephritis or acute rheumatic fever.

It seems probable that other explanations may be offered to account for the regularity of the relatively high antistreptolysin in these conditions.

In the first place, it is possible that certain strains of hemolytic streptococci may call forth the production of greater amounts of antistreptolysin than others. There is, however, no direct evidence that would uphold this view.

In the second place, it is conceivable that the reactive processes of different individuals may not respond either qualitatively or quantitatively in the same way to a similar stimulus. Under such circumstances there might be a wide variation in the frequency with which increased amounts of antistreptolysin appeared in the serum and in the height to which it rose in different forms of streptococcal infections. The differences, under these circumstances, would depend less upon the form of infection than upon the peculiar reaction of the individual to this infection. Such an interpretation would accord very well with the observations made upon the two forms of nephritis designated as A and B. In the first type, high titers were the rule; in the second type, high titers were practically absent, and even when in the Type B cases the titrations were made during and following an exacerbation of nephritis coincident with a severe tonsillitis due to *B. hemolytic streptococci*, an increase in the titer above normal was not observed.

If further studies show that the observations already recorded occur with considerable constancy, they would still further support the view that the formation of antistreptolysin reflects the degree to which the reactive processes of the body respond in certain forms of streptococcal infections or in some diseases following streptococcal infections. It might be found that the response of this particular antibody is exaggerated in erysipelas,\* scarlatina, rheumatic fever and in one form of acute hemorrhagic nephritis. It is to be remarked that in all of these diseases an allergic

\*Spink and Keefer have recently published data to show that the antistreptolysin content of the serum from patients with erysipelas, increased regularly and often rose to a very high titer within the first 20 days after the onset of the illness. High titers persisted for periods of 40 days to 6 months.

factor is considered by many to play a more or less definite part in the pathogenesis and natural history of the process. There is no particular reason, however, to suppose that the allergic factor and the antistreptolysin response are in any way related.

### CONCLUSIONS

1. Repeated determinations of the antistreptolysin content of the serum from normal individuals and from patients suffering from a variety of chronic diseases unrelated to streptococcal infections showed that the titer in the majority of cases fell between 25 and 50 units and rarely exceeded 100 units.

2. In acute infections, due to agents other than hemolytic streptococci, titers above 100 units were encountered a little more frequently (22.7 per cent of 66 cases).

3. Significant increases in antistreptolysin were found in the serum from patients with streptococcal infections. This occurred in order of frequency and in height of elevation in acute tonsillitis and pharyngitis, in miscellaneous infections, in scarlet fever, erysipelas and rheumatic fever. In acute rheumatic fever the antistreptolysin titers were regularly and markedly increased.

4. High antistreptolysin titers were also obtained regularly in one form of acute hemorrhagic nephritis designated as Type A. In a second form, also preceded by streptococcal infections, designated Type B, but with insidious onset, the antistreptolysin titers were rarely above the normal and were in some patients unusually low.

5. Though some correlation could be noted between the antistreptolysin curves and the course of the disease in patients with acute rheumatic fever and with acute hemorrhagic nephritis, this was not very close.

6. Reasons are given for concluding that the occurrence almost constantly of the high antistreptolysin titers that are observed during the course of acute rheumatic fever and acute hemorrhagic nephritis, only indicate the recent occurrence of an infection due to hemolytic streptococci and cannot be used as evidence that these diseases are caused by hemolytic streptococci.

7. There appears to be a difference of reactivity amongst individuals suffering from different forms of streptococcal infections. This leads

to variation in the production of antistreptolysin.

8. Patients suffering from acute rheumatic fever and from acute hemorrhagic nephritis (Type A) appear to be especially prone to the formation of antistreptolysin in comparatively large quantities.

I am indebted to Miss Anne Austin McLanahan for assistance in compiling the tables.

### PROTOCOLS

*Number 11.* Antistreptolysin first determined January 20, fourteenth day of disease. Male, colored, age 32. Admitted J. H. H. January 18, 1933, with pain in chest and swelling of eyes. Always well until December 26, 1932, when he developed erysipelas, complicating epidemic parotitis. During acute attack urine normal. On January 6, trace of albumin in urine; on January 12, swelling of ankles, pain in side, shortness of breath. On admission, anasarca, râles at bases of lungs, slight enlargement of heart, blood pressure 170/100, exudates and hemorrhages in retinae, leukocytes 5,850, albuminuria, cylindruria and marked hematuria; phthalein 65 per cent; N.P.N. 28. Urea clearance 40 per cent normal maximum. Albumin and red blood cells in urine diminished rapidly. Discharged April 5. No edema, blood pressure 120/90, phthalein 80 per cent, N.P.N. 30, urea clearance 91 per cent normal maximum, urine concentrated specimen (Addis count) albumin trace, red blood cells diminished from 21 million to 2.5 million. April 19, 1934, readmitted to hospital with generalized infection by *Bacillus suispestifer*. Recovered after febrile illness of 18 days. During illness urine showed no albumin, no red blood cells, no casts. May 10, 1934, Addis count—specific gravity 1.027, protein 40, red blood cells 300,000, leukocytes 1,000,000, no casts, phthalein 78 per cent, urea clearance 130 per cent normal standard. Blood pressure 110/82 to 95/65.

*Number 20.* Antistreptolysin first determined April 10, fifth day of disease. Male, white, age 16. Admitted to J. H. H. April 8, 1933, with swelling of face and ankles for 3 days. In November, 1932, appendectomy; blood pressure 90/70. Urine normal. On March 16, 1933, scarlatina. On April 5, 1933, 20 days later, swelling of face, ankles, headache, vomiting, frequency of urination and some fever. On admission, third day of illness, temperature 101.6°, anasarca, pleural effusion, hemorrhage in retina, swollen tonsils, presystolic gallop rhythm. Blood pressure 180/115, leukocytes 9,100. Urine smoky, albumin 0.6 per cent, many red blood cells, leukocytes and casts. Cultures from pharynx—B. hemolytic streptococcus + + +, phthalein 80 per cent, N.P.N. 30. Rapid improvement. April 13, blood pressure 136/80, loss of edema with diuresis. Discharged May 28, 1933, symptomatically well. Blood pressure 110/64, phthalein 70 per cent, N.P.N. 33, urea clearance 65 per cent normal standard. Concentrated specimen of urine (Addis count)

normal. December 4, 1933, tonsillectomy. Blood pressure 115/70, phthalein 70 per cent, urea clearance 120 per cent normal standard. Urine concentration 1,016 to 1,030. December 15, 1933, Addis count—specific gravity 1,030, protein 34, red blood cells 1,700,000, casts 3,000. January 25, 1934, blood pressure 110/70. Urine—no albumin, red blood cells, or casts. Well. December 19, 1934, blood pressure 120/74. Urine—no albumin, no casts, no red blood cells. October 9, 1935—urine, no albumin, no casts, no red blood cells.

*Number 23.* Antistreptolysin first determined January 29, tenth day of disease. Female, colored, age 21. Admitted to J. H. H. January 28, 1935. Past health excellent. On January 5, 1935, severe cold and sore throat, followed by cough and swelling of legs and abdomen on January 19, 1935. A few days later nausea, vomiting, headache, nocturia and generalized edema. On examination, pallor, anasarca, exudate over enlarged tonsils, bilateral hydrothorax, enlargement of cardiac dullness, systolic murmur. Blood pressure 158/110, edema of retina, enlargement of liver and spleen and ascites. Urine clear, specific gravity 1,012, acid, albumin +, hyaline and granular casts +++, red blood cells +++, leukocytes +++. Cultures from tonsils *B. hemolytic streptococcus* +++. Phthalein 40 per cent, N.P.N. 30, urea clearance 42 per cent normal standard. Rapid improvement with loss of edema, fall of blood pressure and diminished albuminuria within 10 days. March 9, tonsillectomy without reaction. Discharged March 23, without edema, traces of albumin and a few red blood cells in urine. Blood pressure 90/60, phthalein 90 per cent, urea clearance 78 per cent normal standard. April 3, 1935, the physical examination and urine showed nothing abnormal. Blood pressure 104/66. Urine concentrated specimen—acid, albumin trace, no casts, no red blood cells. November 27, 1935, perfectly well. Blood pressure 104/70. Urine showed no albumin, no casts, no red blood cells.

*Number 19.* Antistreptolysin first determined March 19, second month of disease. Male, white, age 33. Admitted to J. H. H. March 18, 1934, with fistula in ano, localized dermatitis, albuminuria and hematuria. Excessive alcohol. For 2 years, frequent "boils"; for 9 months local infection on left arm. Five months ago, swelling of face; 2 months later blood pressure found to be high and urine to contain albumin and blood. On admission, puffy, face slightly puffy, heart slightly enlarged. Blood pressure 142/90, perianal scars, elevated red granulating lesion 3 × 5 cm. on left arm, cultures from which show numerous *B. hemolytic streptococcus* in pure culture. Hemoglobin 72 per cent, red blood cells 3,800,000; leukocytes 8,100. Urine: specific gravity 1,010, albumin +, red blood cells +++, casts +. Phthalein 48 per cent, N.P.N. 34; urea clearance 62 per cent normal standard. Removal of infection on arm. Discharged June 23, 1934, condition unchanged. Blood pressure 138/95. General health after discharge good, but albuminuria and hematuria persisted. April 24, 1935, no edema, color good. Operative wound healed. Blood pressure 155/105. Phthalein 70 per cent; urea clearance

73 per cent normal standard. Urine: specific gravity 1,025 to 1,037, albumin ++, many red blood cells and casts.

*Number 27.* Antistreptolysin first determined November 14, 1932, third month of disease. Male, white, age 27. Admitted to J. H. H. November 9, 1932, with swelling of face and ankles, vomiting and bloody urine. Rheumatic fever at 15; chronic discharging ear for 20 years. In August, 1932, severe sore throat with fever; two weeks later vomiting and abdominal pain, followed after a week by swelling of face and feet, with bloody urine. Since onset swelling has decreased but bloody urine continues. On admission, no edema, discharging right ear, presystolic gallop rhythm. Blood pressure 138/84. Right kidney palpable. Leukocytes 6,600. Urine: smoky, albumin 0.6 per cent, grossly bloody, many leukocytes and casts. Phthalein 55 per cent. Culture of sputum *B. hemolytic streptococcus*. Discharged in practically the same condition. December 22, 1932—remains symptomatically well, but persistent albuminuria and hematuria. December 5, 1934—blood pressure 128/88, N.P.N. 36; urea clearance 105 per cent normal standard, total blood protein 6.5 grams per liter. Phthalein 75 per cent. Urine: specific gravity 1,007 to 1,023. Urine concentrated (Addis count) protein 147 mgm., red blood cells 6,000,000, casts 700,000. May 1, 1935, symptomatically well. Blood pressure 128/74. Urine: albumin +, hyaline and granular casts +, red blood cells +.

*Number 31.* Antistreptolysin first determined November 14, fourth day of disease. Female, white, age 18. Admitted to J. H. H. November 13, 1932, with backache, fever and bloody urine. Always healthy, no previous infections. Seven days before admission, pain in back; 3 days later, chills, vomiting; next day, headache, anuria and then bloody urine, cough, fever. On admission, temperature 102.4° F., looks ill, face puffy, swollen tonsils. Solidification left lower lobe, bilateral costovertebral tenderness. Blood pressure 110/70. Edema over tibia. Leukocytes 5,000. Urine: albumin +++, red blood cells +++, leukocytes and casts +. N.P.N. 120. Culture from throat: many *B. hemolytic streptococcus*. Sputum: many *B. hemolytic streptococcus*, and pneumococcus Group IV. After first week, improvement in general condition, but appearance of generalized rash lasting 3 days, rise in blood pressure to 140/100; phthalein 30 per cent. By December 5th, gradual decrease in N.P.N. to 36, and of blood pressure to 125/90. Later slow improvement and on discharge February 15, 1933, urine showed only trace of albumin, occasional red blood cells and few casts. Phthalein 80 per cent. In good physical condition, but albuminuria, hematuria and cylindruria persist up to January 1, 1934, when, after severe attack of tonsillitis due to *B. hemolytic streptococcus*, edema appeared, albumin, red blood cells and casts increased in urine. N.P.N. rose to 103 on January 5, and phthalein fell to 4 per cent; blood pressure 103/68. Gradual improvement. Tonsillectomy February 15, followed by increased albuminuria, hematuria and cylindruria. January 8, 1935, symptomatically well. Blood pressure



110/70. Urine: pale, albumin questionable, occasional red blood cells and leukocytes, no casts.

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# THE EFFECT OF EPINEPHRIN ON THE BLOOD LIPOIDS OF NORMAL MAN

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In a recent study of the serum lipoids in diabetic patients by Man and Peters (1), high values for total fatty acids and cholesterol were found in patients with evidences of instability of the vasomotor system. In this department high values have been observed in patients with manic-depressive psychosis. This latter group also displayed evidences of marked vasomotor disturbances along with other signs of a disorder of the sympathetic nervous system. The reports by some investigators (2, 3), that epinephrin when injected into an animal may elevate the fatty acids and cholesterol of the blood suggest that this hormone may have been an important factor in producing the lipemias which have been found in association with the disturbances of the vasomotor system in the two groups of patients.

Certain other workers (4, 5, 6) have reported a fall in fatty acids or in cholesterol after the injection of epinephrin; others (7, 8, 9) have stated that there is no change. Most of these investigators have employed the older colorimetric and nephelometric methods for the measurement of cholesterol and fatty acids. These methods have been proved to be unreliable and subject to large limits of error in even the most skilled hands and have been abandoned for the most part by their original supporters. The various methods for the measurement of serum fatty acids have been discussed by Bloor in 1928 (10) and by Man and Gildea in 1932 (11). Evaluations of cholesterol methods have been presented in the papers of Mühlbock and Kaufmann in 1931 (12), and Man and Peters in 1933 (13). Recently Page, Kirk, Lewis, Thompson and Van Slyke, 1935 (14), have published a comprehensive review of lipid methods.

On account of this uncertainty in method only the experiments which have been carried out with the oxidative or the titrimetric methods for fatty acids and the digitonin precipitation methods for cholesterol will be discussed. The work done

with the Stewart and White method has not been considered because of the serious errors in this technique which have been described by Long and Venning, 1932 (8), and Man and Gildea, 1932 (11).

Page and Pasternak (4) administered epinephrin to rabbits at 15 minute intervals for 4 hours and at the end of this period observed a fall in the fatty acids, cholesterol and lipid phosphorus of their serum. These authors have not considered the possibility that the rabbits were in such an excited state at the beginning of the experiment that they were already experiencing the maximum effects of epinephrin. Jones and Fish (2) reported that they found rabbits unsatisfactory for experiments with epinephrin. Rony and Ching (9) observed no change in the blood of dogs after the administration of epinephrin. Long and Venning (8) using a titrimetric method similar to that of Stoddard and Drury also observed no change in dogs. In a brief article Miller (15) reported that slight and variable changes were produced in the lipoids of serum of unanesthetized dogs, but that if the dogs were anesthetized before injection of the drug a 60 per cent rise in the phospholipoid fraction of the serum fats occurred while the other lipoids were not significantly changed. Jones and Fish administered 0.5 ml. of a 1/1000 solution of epinephrin subcutaneously to 13 normal people and found an elevation in the fatty acids of the serum one-half to one hour later. The maximum rise was 69 mgm. per cent, the average 30 mgm. per cent and the minimum in one subject was 12 mgm. per cent. At the end of two hours the fatty acids had returned practically to the level obtaining before injection. All of the studies on the effects of epinephrin on serum cholesterol have been carried out with colorimetric methods, and the results are as conflicting as are those on fatty acids.

The discrepancies in the results of various studies may be due to the following factors. The

110/70. Urine: pale, albumin questionable, occasional red blood cells and leukocytes, no casts.

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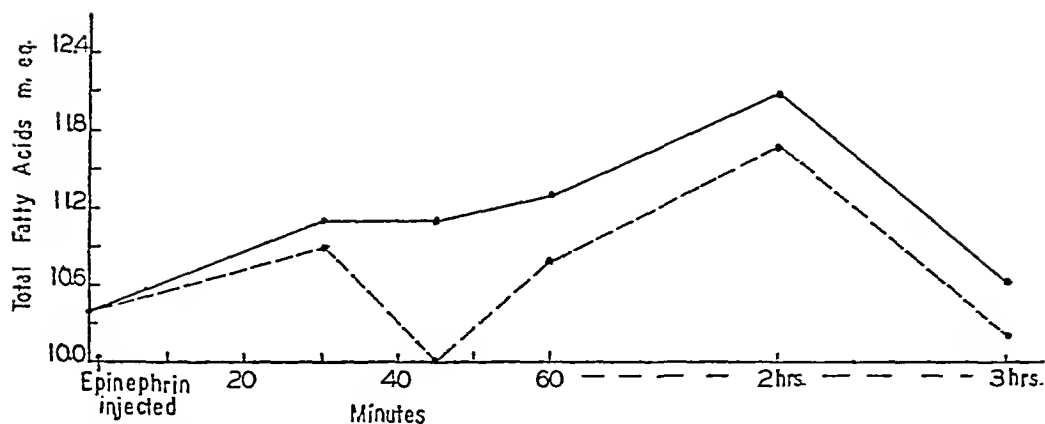


FIG. 2. THE CHANGES IN SERUM FATTY ACIDS FOLLOWING THE INJECTION OF EPINEPHRIN

The broken line represents the apparent changes in fatty acids when not corrected for hemodilution, and the solid line the values after correction.

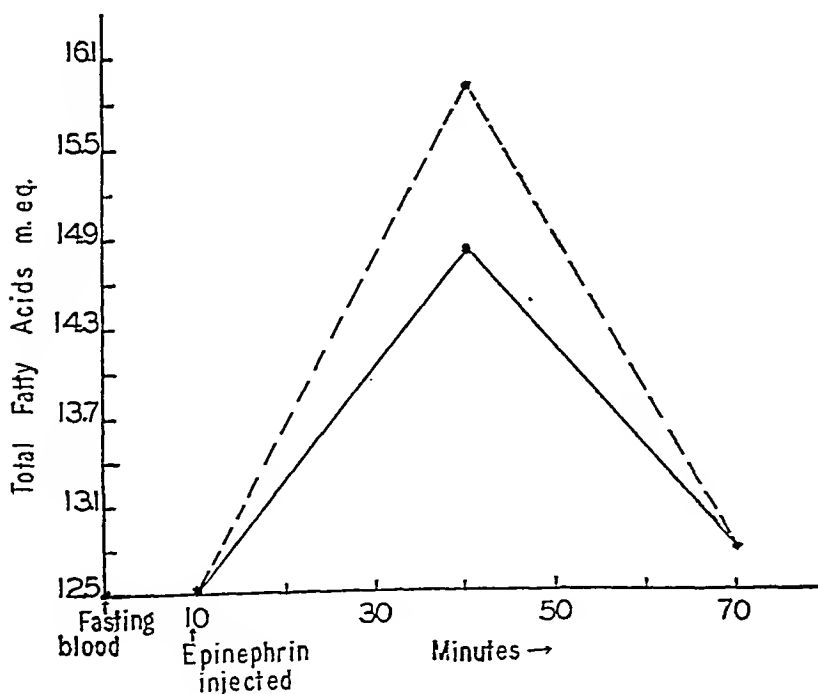


FIG. 3.

The broken line represents the apparent changes in fatty acids after the injection of epinephrin when no correction has been made for hemoconcentration, and the solid line represents the corrected values for the fatty acids.

methods employed in measuring the lipoids may account for some failure in agreement. The possibility that the various animals which have been used may react to epinephrin in different ways constitutes a factor which has rarely been considered. And finally the rôle which hemoconcentration or dilution alone may play in producing apparent changes in the blood lipoids has been neglected by previous investigators.

The serum fatty acids were determined by the titrimetric methods previously described by Man and Gildea, 1932 (11), the phospholipoid fraction was measured as phosphorus (13) and the cholesterol by precipitating, washing and weighing the isolated cholesterol digitonin according to the technique of Man and Peters, 1933 (13).

The total proteins of the serum were measured by the technique of Bruckman, D'Esopo and Peters, 1929-30 (16), as a means of determining the extent of change in the concentration of the blood serum which might occur.

vein sometime between 8:00 and 10:00 a.m. The epinephrin solution in a dilution of 1/1000 as supplied in ampoules by Parke Davis was administered by intramuscular injection. The site of the injection was subsequently massaged for 1 to 2 minutes. The first three subjects were given 1 ml. of the epinephrin solution, the others, except where repeated doses were used, received only 0.5 ml. In most cases samples of blood were taken 30 minutes, 1 hour and 2 hours after the injection of epinephrin. In three subjects samples of blood were obtained at intervals of 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours and 3 hours after administration of the drug.

The data are presented in charts. Figures 1, 2 and 3 are self-explanatory. In Figure 4 the maximum amount that the fatty acids have risen in each subject studied has been plotted in arithmetical terms along the ordinate and the arithmetical changes in blood sugar, systolic blood

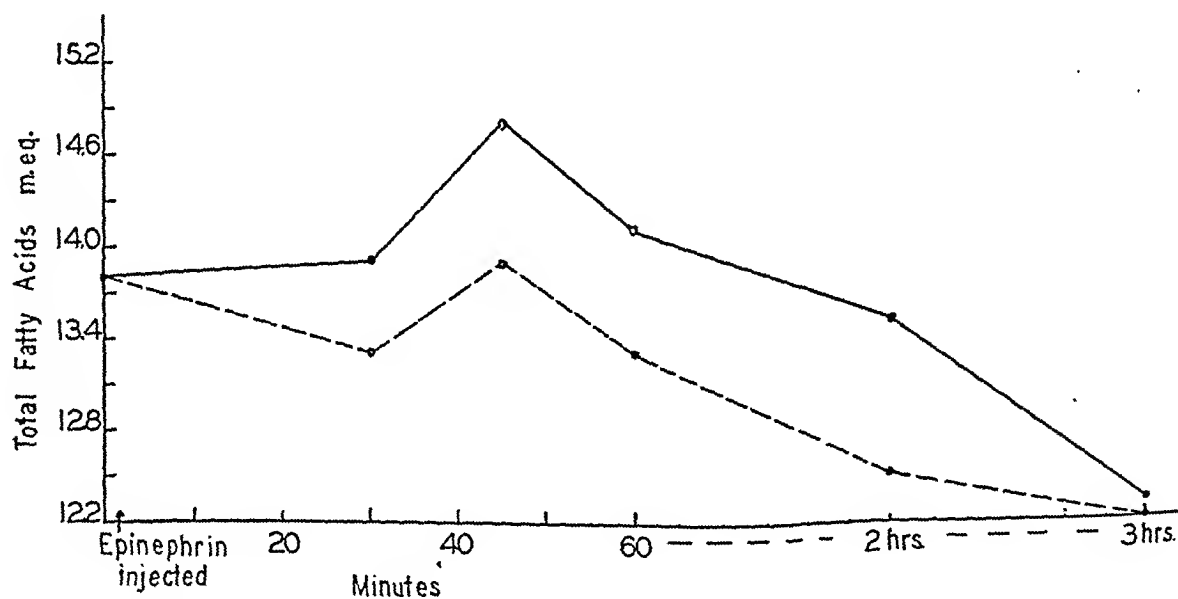


FIG. 1. THE CHANGES IN SERUM FATTY ACIDS FOLLOWING THE INJECTION OF EPINEPHRIN.

The broken line represents the apparent changes in fatty acids when not corrected for hemodilution, and the solid line the values after correction.

Eleven of the subjects utilized in these studies were either students or members of the staff of the university. Five other subjects who were patients were studied because they showed symptoms of severe vasomotor instability, but were free from any demonstrable organic disease. All subjects were in the postabsorptive state and the first samples of blood were taken from an arm

pressure and cholesterol have been plotted on separate scales along the abscissa. The cross represents the relationship of the maximum changes in the fatty acids and blood sugar for each subject. In a similar fashion the circle represents the relationship of fatty acids to systolic blood pressure and the square the relationship of fatty acids to cholesterol.

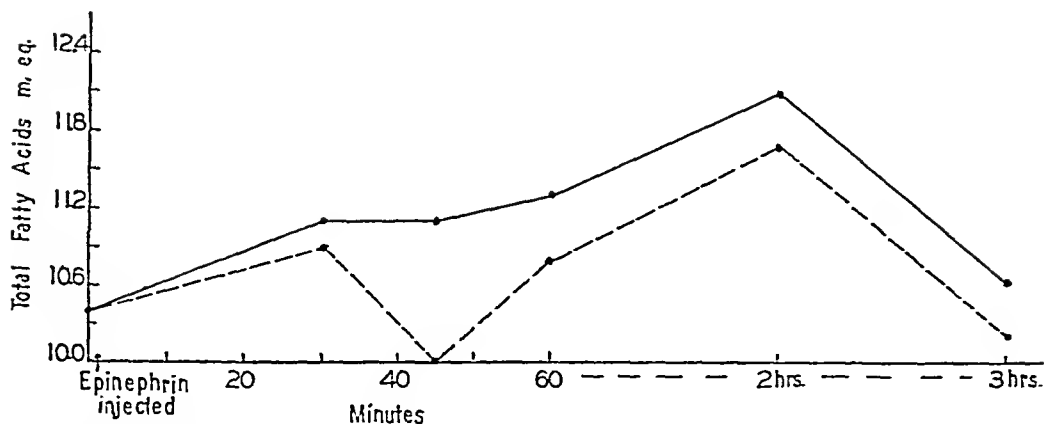


FIG. 2. THE CHANGES IN SERUM FATTY ACIDS FOLLOWING THE INJECTION OF EPINEPHRIN

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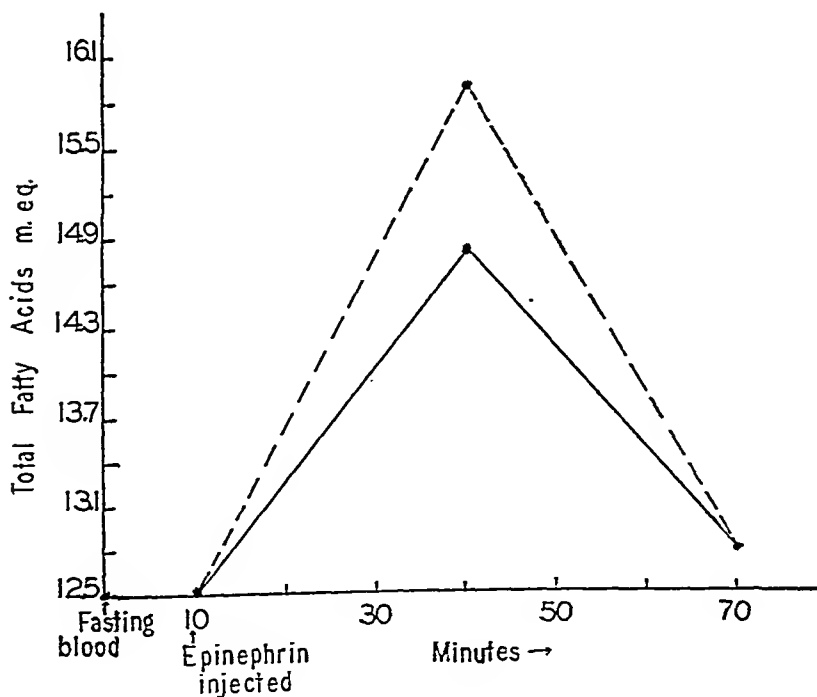


FIG. 3.

The broken line represents the apparent changes in fatty acids after the injection of epinephrin when no correction has been made for hemoconcentration, and the solid line represents the corrected values for the fatty acids.

methods employed in measuring the lipoids may account for some failure in agreement. The possibility that the various animals which have been used may react to epinephrin in different ways constitutes a factor which has rarely been considered. And finally the rôle which hemoconcentration or dilution alone may play in producing apparent changes in the blood lipoids has been neglected by previous investigators.

The serum fatty acids were determined by the titrimetric methods previously described by Man and Gildea, 1932 (11), the phospholipoid fraction was measured as phosphorus (13) and the cholesterol by precipitating, washing and weighing the isolated cholesterol digitonin according to the technique of Man and Peters, 1933 (13).

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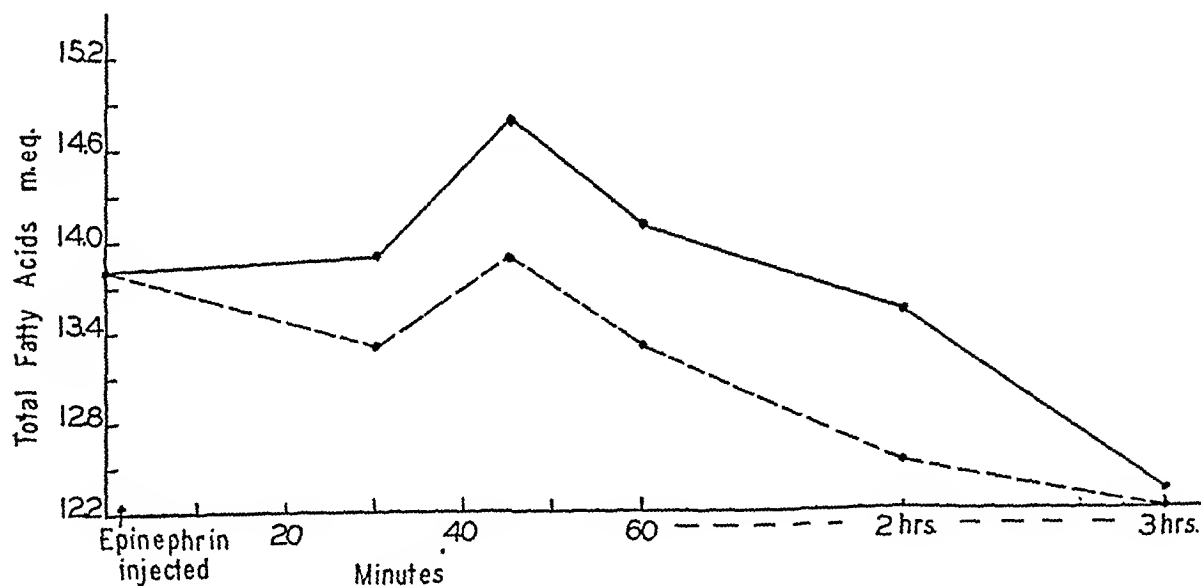


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#### DISCUSSION

The above experiments indicate that single injections of epinephrin in amounts sufficient to elevate the blood sugar and blood pressure and to cause a generalized body tremor, produce consistent but relatively slight rises in the serum fatty acids when a correction for the effect of serum dilution or concentration is made. The fact that there occurred in 4 of 11 normal subjects a hemodilution, which in two was sufficient to produce an apparent decrease in the lipoids, may explain the reports of some observers of a fall in lipoids after epinephrin.

When the factor of hemoconcentration is considered the absolute changes in lipoids are diminished to a point where the increases in only 9 of the 16 subjects fall outside the maximum variations that may occur in samples taken at hourly intervals when epinephrin is not administered. It is noteworthy that in the epinephrin experiments the fatty acids always rose or remained unchanged. The same was true for cholesterol with 3 exceptions. On the other hand the serum lipoids may either rise or fall in the hourly variations which occur in the normal subject in the postabsorptive state. As a control the lipoids were determined at 9:27, 10:27 and 11:27 a.m. in Subjects E. F. G. and E. B. M. while they were in the postabsorptive state and without the administration of epinephrin. In the case of E. F. G. the total fatty acids deviated from the initial determinations by  $+0.7$  milliequivalents and the cholesterol by  $+3$  mgm. per cent while in the case of E. B. M. the fatty acids rose  $0.4$

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These experiments illustrate again the usually recognized variability in action and effects of epinephrin even in a normal group of subjects. There was not even a consistent relationship between the changes in blood sugar and blood pressure and the degree of change in the lipid constituents. This marked variability in the action and effect of epinephrin on "normal" subjects makes it extremely precarious to use its action to test the adequacy of physiological functions in patients who are suspected of liver dysfunction, disorders of the sympathetic system and other diseases.

The administration of  $1$  ml. of the epinephrin solution did not produce any greater changes in the lipoids of the serum than did  $0.5$  ml. although the larger amounts resulted in more marked and prolonged symptomatic discomfort. For this reason it does not seem probable that larger doses of epinephrin would be more effective in changing the level of blood lipoids. Repetition of the epinephrin also did not produce a greater change in the lipoids than did a single injection. This observation agrees with the prolonged experiments on animals reported by Page and Pasternak (4) and by Long and Venning (8).

In view of the relatively small changes which occurred in these experiments, epinephrin cannot be implicated as the factor responsible for the high lipoids that have been observed in patients with symptoms of vasomotor instability.

#### CONCLUSIONS

The subcutaneous injection of epinephrin in 11 normal subjects and 5 patients in amounts sufficient to increase the blood sugar and blood pressure, produced in the subsequent 2 hours, a



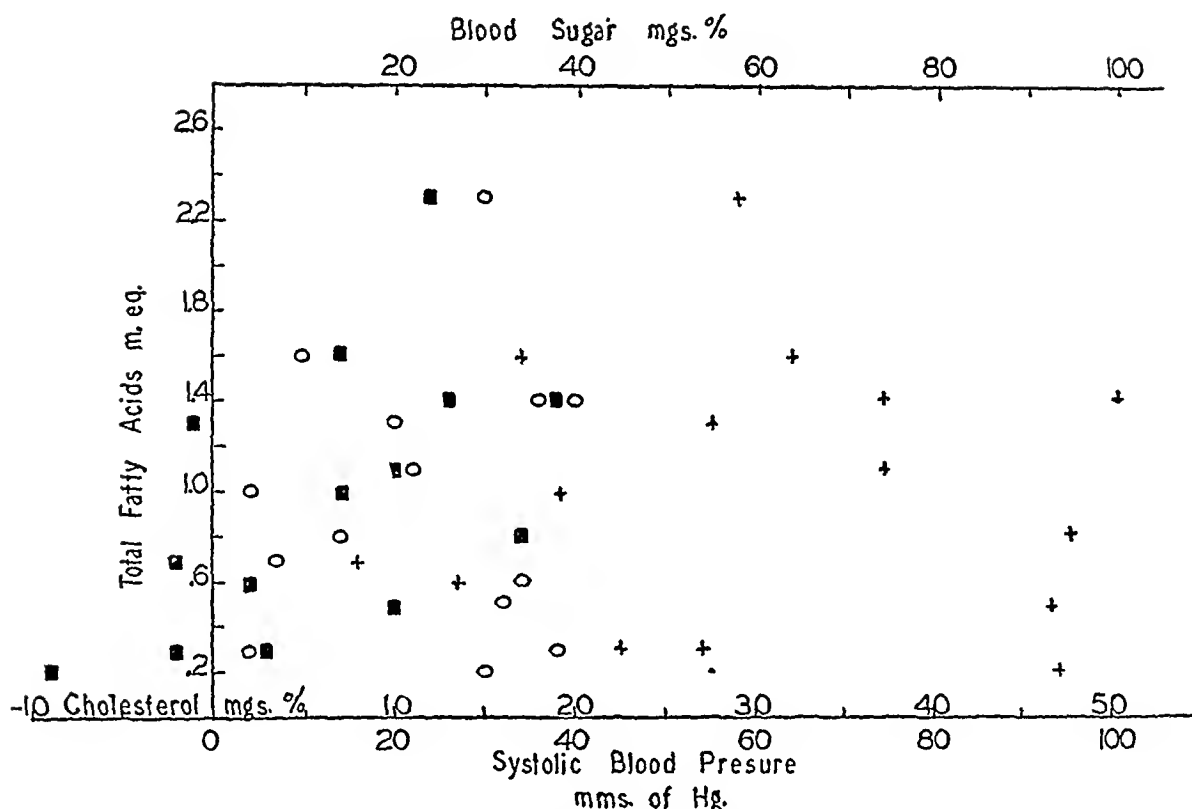


FIG. 4. A COMPARISON OF THE ARITHMETICAL CHANGES OF THE FATTY ACIDS FROM THE POSTABSORPTIVE LEVEL, WITH ARITHMETICAL CHANGES IN BLOOD SUGAR (+), BLOOD PRESSURE (O), AND SERUM CHOLESTEROL (■).

#### OBSERVATIONS

The total fatty acids rose 1 to 2.3 milliequivalents above the postabsorptive level in 9 of the 16 subjects, more than 0.5 in 4, and less than 0.5 in 2. The cholesterol rose 10 to 19 mgm. per cent in 6 of the subjects, 7 mgm. in 2, remained unchanged in 5, and fell 9 mgm. per cent in 2. The phospholipid deviations in most instances were not greater than the limits of error for the method. In 8 of the studies there was a fall, and in the other 8 a rise. The changes in lipoids took place in the first hour. At the end of 2 hours lipoids were at approximately the postabsorptive level. All of the values were corrected for changes in concentration of the serum as measured by the change in total proteins. Justification for this procedure is based on previous observations that serum lipoids and proteins change proportionally with rapid alterations in blood concentration (17, 18). The proteins increased 0.11 to 0.61 gram per cent above the postabsorptive level in 9 subjects and fell 0.12 to 0.52 gram per cent in 5. The deviations were less than  $\pm 0.1$  gram per cent, the limit of error for the method,

in 2 subjects. These changes in concentration were sufficiently great in many instances to produce a marked difference between the corrected and uncorrected lipid values. In 2 studies correction for this factor revealed an absolute increase in fatty acids although dilution of the serum was sufficient in amount to produce an apparent fall (Figure 1). In other instances apparently small elevations in fatty acids were increased by correction for hemodilution (Figure 2). In the subjects where hemoconcentration occurred correction acted in the reverse direction and diminished the apparently large elevations in fatty acids (Figure 3). In Figure 4 the distribution of the crosses indicates that although a marked rise in blood sugar occurred in every subject, there was a striking lack of relationship between the magnitudes of the increases in fatty acids and blood sugar. The scattered distribution of the circles demonstrates that there was little relationship between the amount of the increase in fatty acids and systolic blood pressure. The arrangement of the squares indicates, however, that the changes in cholesterol although irregular were similar to those of the fatty acids.

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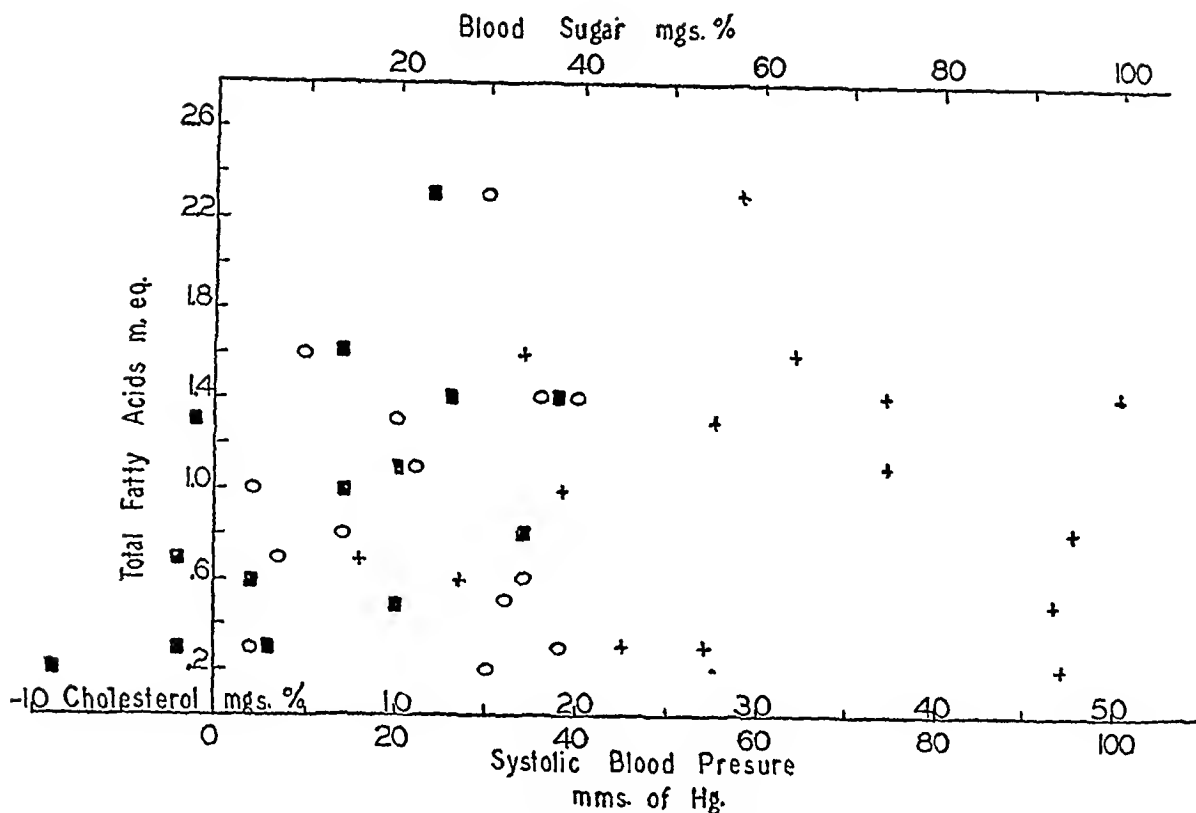


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#### CONCLUSIONS

The subcutaneous injection of epinephrin in 11 normal subjects and 5 patients in amounts sufficient to increase the blood sugar and blood pressure, produced in the subsequent 2 hours, a

moderate but consistent elevation in the level of serum fatty acids.

In 9 of the subjects the total fatty acids were increased by as much as 1 to 2.3 milliequivalents, and in 5, cholesterol increased 10 to 19 mgm. per cent. These values were corrected for changes in the concentration of the serum.

Hemoconcentration, as measured by total proteins of the serum, occurred in 9 subjects, while 5 showed hemodilution. The importance of this factor in changing the apparent level of serum lipoids has been discussed.

It has been pointed out that the variability of the responses of the different normal subjects to epinephrin was too great to warrant the use of this drug as a test of physiological function in patients.

No evidence was obtained from these experiments to suggest that epinephrin might play an important part in determining the level of blood lipoids.

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# OBSERVATIONS ON THE FATE OF SODIUM SULFATE INJECTED INTRAVENOUSLY IN MAN<sup>1</sup>

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The present work was carried on in an attempt to investigate the fate of sodium sulfate injected intravenously in man as regards its diffusion into body fluids, its effect on some of the electrolytes of blood, and its excretion. Before these subjects can be discussed, however, certain considerations must be given to the distribution of endogenous sulfate, and some experimental facts and observations should be quoted.

The concentration of inorganic sulfate in normal human serum has been studied by several investigators. Their findings, reviewed recently (17), range from 0.1 to 3.3 mgm. of sulfur per 100 cc. Using Cope's technique (2), we have found, for ten determinations on eight normal subjects, an average of 0.66 m.eq. per liter of total serum, with figures ranging from 0.37 to 0.92 m.eq. (1 m.eq. per liter = 1.6 mgm. of sulfur per 100 cc.). These figures are in exact agreement with those of Loeb and Benedict obtained gravimetrically on 10 cc. of serum (21). In pathological conditions, especially in chronic nephritis, this level may rise very high (5, 6, 8, 21, 29). According to Macy (22), it varies but little in the normal person in the course of the day.

There is still much doubt concerning the inorganic sulfate content of red cells. Reed and Denis (27) claim that blood inorganic sulfate is almost entirely confined to plasma; Cuthbertson and Tompsett (5), that one fifth is in the cells. We have confirmed Øllgaard's observation (23) that analytical methods involving benzidine are not suitable for corpuscles or whole blood. No accurate information is available in the literature. For the present investigation this uncertainty seems immaterial. More important is the question of the permeability of the red cells to added sulfate. It has been shown (30, 31) that red cells are practically impermeable to sulfate added *in vitro*. In experiments conducted in this lab-

oratory it has been observed that human red cells become permeable to a variable degree only when added sulfate is left several days in contact with blood, or when blood is subjected to an extreme saturation with CO<sub>2</sub>; in other words, under circumstances that are never realized in physiological or pathological conditions. That, *in vivo*, red cells should be rapidly permeable to inorganic sulfate seems, therefore, improbable.

Since no determinations of the inorganic sulfate content of transudates seem to have been published thus far, some figures obtained by us are given (Table I). Too much stress must not

TABLE I

Distribution of chloride and sulfate between serum and transudates

Diagnosis	SO <sub>4</sub>		Cl		Proteins	
	Per liter of fluid	Per liter of serum	Per liter of fluid	Per liter of serum	Per 100 cc. of fluid	Per 100 cc. of serum
(Pleural fluid)	m.eq.	m.eq.	m.eq.	m.eq.	grams	grams
1. Rheumatic fever, extreme heart failure...	1.61	2.05	105.0	109.0	1.7	5.0
2. Acute anuria.....	3.04	4.19	109.9	102.8	1.5	
3. Chronic nephritis.....	2.42	2.51	114.0	107.2		
Same, two weeks later.....	2.98	2.96				
(Peritoneal fluid)						
4. Liver cirrhosis.....	.84	.71	111.6	102.5	1.1	5.7
5. Liver cirrhosis.....	.74	.67	100.2	93.8	1.4	6.5
6. Liver cirrhosis.....	.60	.64	99.6	91.8	0.9	5.8
7. Polyserositis.....	.80	.63	103.6	101.5	4.2	6.5

be placed on them, since most were not obtained in the postabsorptive state, and often blood and fluid were drawn a few hours apart. It is evident that they do not show a constancy of distribution comparable to that of chloride. For the present purpose, the figures suffice to show that endogenous sulfate is, roughly speaking, equally distributed between serum and transudates, and presumably also extracellular tissue fluids. This is supported by some figures given by Hayman (15), which show that the concentrations of inorganic sulfate in ultrafiltrates of serum and in native serum are the same. In three determina-

<sup>1</sup> Part of the expense of this investigation was defrayed by a grant from the Ella Sachs Plotz Foundation.

tions we found that sulfate was decidedly lower in spinal fluid than in the corresponding sera.

#### EXPERIMENTAL

Sulfate was in most cases injected with thiocyanate in order to afford a means of comparison for the extent and velocity of its diffusion. Doses of  $\text{Na}_2\text{SO}_4$  ranged from 1.3 to 19 grams. Thiocyanate was injected as  $\text{NaCNS}$  in doses of 1 to 1.5 grams added to the sulfate. The solution was made up to about 4 per cent with freshly distilled water, boiled 10 minutes for sterilization, and injected at the rate of 10 to 20 cc. per minute. In some cases  $\text{KCNS}$  was taken perorally (1 to 1.5 grams) the evening before. The subjects were in the postabsorptive state; they were the two authors, in good health, convalescent patients, and a few nephritics in a state of advanced renal failure. No meal, except a little coffee or fruit juice in some cases, was taken during the experiment. No toxic effects were noted.

All determinations were made on venous serum, the blood being drawn without stasis and allowed to clot under oil. Inorganic sulfate was determined after Cope (2), the same method proving quite convenient for dilute urine; thiocyanate after Crandall and Anderson (4);  $\text{CO}_2$  with the Van Slyke manometric apparatus (25); total base and sodium in serum after Hald (14); urine sodium after Butler and Tuthill (25); serum chloride after Hald's modification of the Volhard-Harvey method (25);  $\text{O}_2$  capacity by the Van Slyke and Hiller carbon monoxide method (25); proteins by macro-kjeldahl (25), red cell volume with Daland hematocrit tubes.

Recently Hoffman and Cardon (17) have claimed that all analytical methods involving benzidine previously published, including Cope's, are quite misleading, the results obtained being always far too high, sometimes as much as 300 per cent in nephritic sera. The objections raised by these authors certainly lack convincing evidence. That phosphate does not interfere practically with the procedure has been shown several times (2, 5, 18, 23). The values we have obtained for normal sera are exactly comparable to those found by Loeb and Benedict (21) (from 0.4 to 1.0 m.eq., average 0.7) in thirteen determinations made on 10 cc. of serum with the  $\text{BaCl}_2$  gravimetric method. This is also the range of figures obtained by Øllgaard (23) (from 0.7 to 1.5 mgm. of sulfur per 100 cc. in twenty determinations), who used a procedure involving benzidine sulfate titration with

$\text{BaCl}_2$ ,  $\text{MgCl}_2$  being added in order to eliminate possible interference of phosphate. The high figures we have often found in nephritic cases are quite in agreement with those of Loeb and Benedict. We can not certify that the precipitate that forms in Cope's method is strictly pure benzidine sulfate; but the fact that we have been able to recover small amounts of  $\text{Na}_2\text{SO}_4$  added to normal and nephritic sera with an error no greater than 4 per cent should be sufficient proof that the method is quite suitable for the experiments here recorded.

The original method of Cope has been slightly simplified by the substitution for the Rehberg burette of a 0.15 ml. microburette of the ordinary type, while the titration tube is heated directly with a microburner instead of a steam-jacket.

When checking the method on theoretical solutions of  $\text{Na}_2\text{SO}_4$ , containing from 0.15 to 2.25 m.eq. per liter, it was observed that a certain constant loss occurred, which was independent of the amount determined. The minimum for this loss was equivalent to 0.002 or 0.003 ml. of 0.02 N  $\text{NaOH}$ , but it would sometimes rise to 0.010 ml., the striking feature being that the loss was always exactly the same for all the tubes of one batch of determinations. The conditions of the whole procedure were always kept rigorously alike, and it has not been possible thus far to determine the exact source of error. The acetone and trichloroacetic acid used were sulfate free. When distilled water was subjected to the whole procedure the titration did not differ from the titration on distilled water alone; i.e., the same negative correction of a few c. mm. only of  $\text{NaOH}$  solution was necessary for the end-point of phenolphthalein. Varying the length of time the tubes were left standing before centrifugation, from 0 to 1.5 hours, did not seem to make any difference. It is suggested that a variable amount of benzidine sulfate, comprising that originally contained as impurity in the benzidine, plus a small amount of the benzidine sulfate formed in the reaction, remains in solution and can not be recovered. For the determinations given in this paper, a correction of + 0.006 ml. of  $\text{NaOH}$  was habitually used to offset this loss. Whatever may be the cause of the error, it is too small to affect in any appreciable fashion the results presented here.

#### *Sulfate excretion*

For the purposes of these experiments it is important to know that exogenous sulfate acts as a foreign substance and can be recovered *in toto* from urine. This has been shown to be true for thiocyanate (26). The fact that sulfate is apparently a natural waste product makes it unlikely that it should undergo chemical transformation; any considerable increase of phenol conjugation is unlikely under the conditions of these experiments (16). Consideration must, of course, be given to the excretion of endogenous sulfate

which, in the fasting subject, according to Macy (22) amounts to about 1 m.eq. per hour. This may rise to as much as 3 m.eq. per hour in the course of the day in persons receiving meals, paralleling rather closely the urinary nitrogen, since urinary sulfate is largely derived from protein (25). It may also be increased by profuse diuresis. In these experiments, however, feedings containing protein were avoided and diuresis was never evident. It has, therefore, been assumed that endogenous sulfate excretion regularly amounted to 1 m.eq. per hour. On this basis, in Experiment 3b, Table II, at the end of 6.5 hours,  $51.7 - 6.5 = 45.2$  m.eq. of exogenous sulfate had been recovered in the urine after the injection of 47.3 m.eq., a reasonable agreement. Serum sulfate, meanwhile, had returned to its original level.

A glance at Table IV shows that sodium and sulfate were excreted in approximately equivalent amounts without any considerable excess of water or chloride. This may be taken as contributory evidence that the salt is treated as a single foreign substance and subjected to no metabolic transformations.

#### *Sulfate concentration in serum*

Serum sulfate levels after injection, recorded in the first column of Table II, decrease rapidly in normal, slowly in nephritic subjects. This is illustrated by Figure 1, in which the concentration of exogenous sulfate in serum (concentration observed minus fasting concentration) in all experiments of Table II have been plotted logarithmically against time. It has been observed in dogs (15) that the rate of excretion of injected sulfate is proportional to the concentration of sulfate in serum. Dominguez and Pomerene (11) have shown that the decreasing serum level and excretion rate of exogenous creatinine both yield satisfactory exponential curves. That in man sulfate is excreted in the same fashion seems likely from Experiments 2, 3b, 5, 6, and 8a plotted logarithmically in the figure, since all yield three points approximately on a straight line. Experiments 1, 3a, and 6 show that in the first ten or twenty minutes after the injection there is a sharp drop. (In Experiments 1 and 3a, blood was drawn one minute after the end of the injection, and from the other arm; in Case 6, from the same arm,

TABLE II

*The distribution of intravenous SO<sub>4</sub>*

Experiment number	Time from beginning of injection	Serum SO <sub>4</sub>	Volume of distribution of		Urinary SO <sub>4</sub>
			SO <sub>4</sub>	CNS	
	minutes	m.eq. per liter	liters	liters	m.eq.
1. Normal male. Body weight 52 kgm. 19 m. eq. injected * in 2 minutes	0	0.3			
	3	3.0	8.2	10.5	
	4	2.6	9.6	10.4	
	5	2.6	9.6	11.9	
	15	2.1	12.2	13.1	
	180	0.8			
2. Normal female. Body weight 88 kgm. 64.9 m.eq. injected * in 5 minutes	0	0.5			
	20	4.4	16.6	16.8	
	40	4.0		18.1	
	125	2.4		19.7	
3a. Normal male. Body weight 85 kgm. 39.7 m.eq. injected * in 4 minutes	0	0.7			
	6	5.3	8.8	12.1	
	9	3.9	12.4	14.6	
	20	3.4	14.7	15.7	
b. Body weight 85 kgm. 47.3 m.eq. injected in 6 minutes	0	0.9			
	35	2.9	16.7		14.6
	135	1.9	18.0		17.0
	255	1.2			10.4
	390				9.7
c. Body weight 85 kgm. 41.8 m.eq. injected * in 8 minutes	0	0.8			
	15	3.7	12.2	13.9	6.7
	45	2.5	15.3	16.9	9.9
4. Normal female. Body weight 54 kgm. 50.7 m.eq. injected in 8 minutes	0	0.9			
	25	5.2	9.3		11.0
	120	2.2			30.0
	180				
5. Normal male. Body weight 65 kgm. 29.9 m.eq. injected ‡ in 5 minutes	0	0.9		17.0	
	15	2.8	11.7		7.3
	45	2.1	14.3		6.3
	105	1.5			5.2
6. Female, chronic nephritis. Body weight 44 kgm. 19 m.eq. injected * in 5 minutes	0	2.2			
	6	4.4	8.6	11.3	
	20	3.6	13.6	14.5	
	65	3.6	13.6	14.8	
	360	3.4	15.8	15.8	
7. Female, chronic nephritis, edema. Body weight 49 kgm. 30.5 m.eq. injected *	0	2.4			
	20	4.6	13.9	17.0	
	185	4.0	19.1	22.0	
8a. Male, chronic nephritis. Body weight 54.4 kgm. 28.1 m.eq. injected ‡	0	2.5		16.8	
	25	4.6	13.1		
	50	4.4	14.8		
	100	4.1			
b. Body weight 56.8 kgm. 40.8 m.eq. injected ‡	0	1.8		17.9	
	50	4.2	17.0		
	100	3.9			

\* NaCNS injected at the same time.

‡ KCNS given perorally 12 hours earlier.



after about 30 seconds.) This is evidence that the substance takes an appreciable time to reach its final distribution between blood and tissues.

The three curves obtained from Subject 3, after injection of comparable amounts of sulfate, follow

diffuses approximately evenly into all the fluids except the water in tissue cells; this volume of fluid represents a definite, and, in the normal individual, little changing entity (19). It may be calculated by dividing the amount of thiocyanate

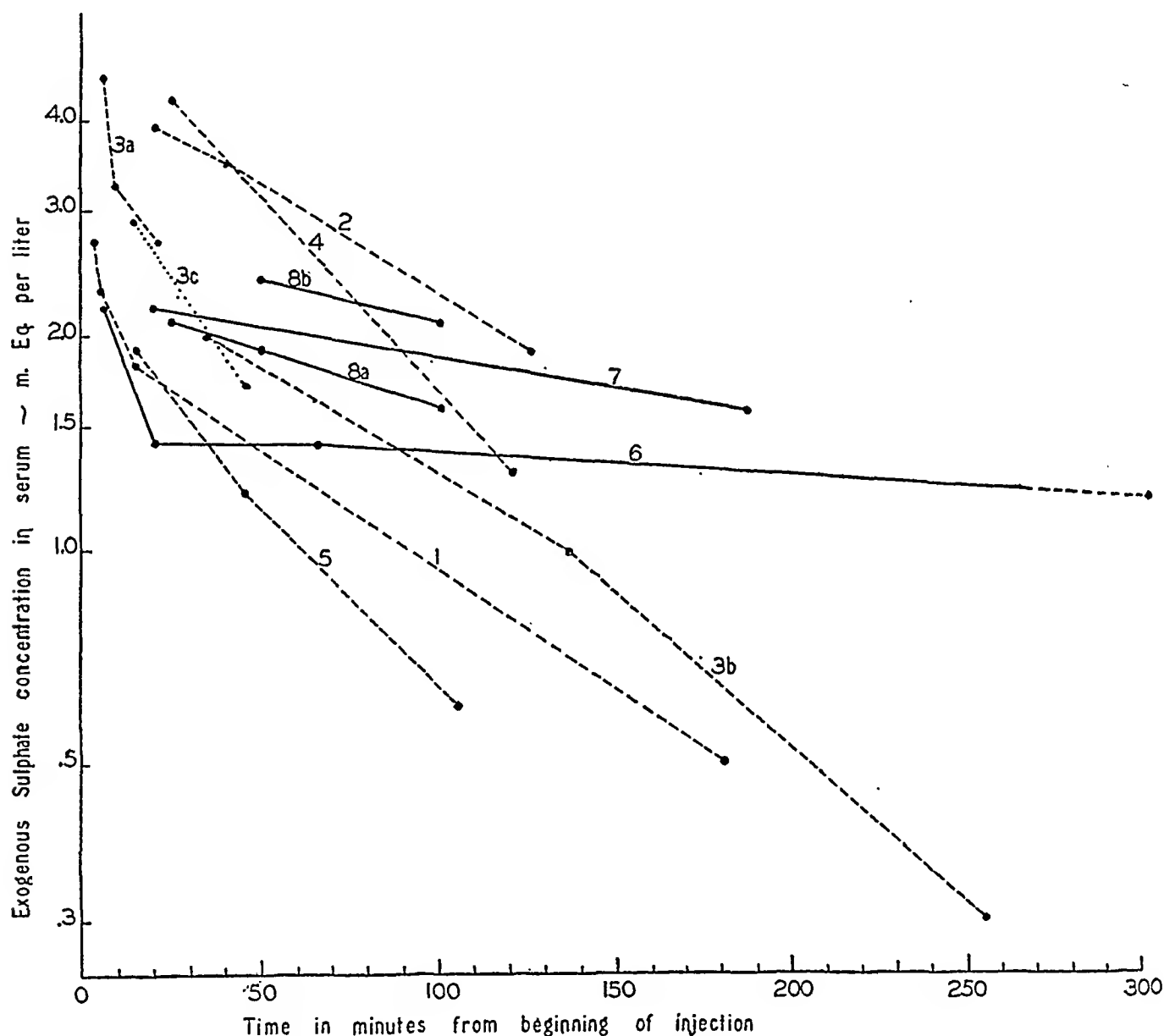


FIG. 1. THE RATE OF DISAPPEARANCE OF INJECTED SULFATE FROM SERUM IN NORMAL SUBJECTS (-----) AND IN PATIENTS WITH CHRONIC NEPHRITIS (—).

each other closely, showing that the reaction of the same individual to the repeated procedure is quite constant. Curves 6, 7, 8a and 8b, obtained from nephritics, are much less steep than the normal ones.

#### *Sulfate distribution*

There are strong reasons for believing that when thiocyanate is introduced into the body, it

injected, minus the amount excreted, by the concentration found in serum.

To apply the same calculation to injected sulfate is a little more complicated, since it is necessary to deduct, from the serum level observed, the fasting level, and, from the sulfate excreted, a certain amount for endogenous excretion. In the experiments recorded here, this amount was assumed for reasons already given, to be 1 m.eq.

per hour. In some cases urinary excretion was not determined.

In Table II, it appears that distribution figures for sulfate are in rather close agreement with the corresponding thiocyanate figures, but always somewhat lower. It should be noticed that only in the experiments in which sulfate excretion was determined and taken into account in the calculation are the sulfate distribution figures exact. In the other experiments, they are too large, the error becoming greater as time elapses. Nevertheless, even as they are given here, they do not reach the thiocyanate figures as late as 15 or 20 minutes after the injection in normal cases, and much later in nephritic subjects, in which renal excretion must have been considerably retarded (especially in Experiment 6). Consequently, the extent of sulfate distribution in all cases, although it follows that of thiocyanate in its progressive increase, always falls short of it by a certain proportion. The water of red cells, into which thiocyanate only penetrates, would account for part of the difference. This conclusion holds only, of course, if the distribution ratios between serum and extravascular fluids are always the same for  $\text{SO}_4$  and CNS, which is only approximately true. Roughly speaking, sulfate and thiocyanate must diffuse into approximately the same volume of fluid. That changes in this volume are reflected by changes in the distribution of both salts is illustrated by Experiments 8a and 8b, made 6 days apart; an increase of 2.4 kgm. in body weight, induced in the meantime by forcing NaCl and water, is accompanied by an increase in the distribution figures of both thiocyanate and sulfate.

#### *Effects of large injections of sulfate*

It has been shown (4) that thiocyanate, when introduced into the body, diffuses into a fraction of fluid which represents on the average 24 per cent of the body weight, in man. This is the fraction of the body fluid which has been called above the volume of distribution. There is reason to believe that it consists chiefly of the extracellular or interstitial portion of the water of the body (19). That injected sulfate diffuses through approximately the same portion as thiocyanate is evident from the experiments just discussed. If this is extracellular fluid it should

contain also the major portion of the sodium and chloride of the body (5a, 14a). As far as thiocyanate, sulfate and chloride are concerned this deduction finds support in certain data collected from the literature which are presented in Table III. In the first column the ratio, tissue Cl:

TABLE III

*Distribution of chloride, thiocyanate and sulfate in dogs*

	$\frac{\text{Tissue Cl}}{\text{Blood Cl}}$	$\frac{\text{Tissue CNS}}{\text{Blood CNS}}$	$\frac{\text{Tissue SO}_4}{\text{Serum SO}_4}$
Lung.....	.77	.81	.80
Kidney.....	.84	.53	.86
Heart.....	.40	.61	.35
Liver.....	.45	.42	.47
Muscle.....	.22	.13	.08

blood Cl, has been calculated from tissue analyses of Cameron and Walton (1) with the assumption that blood Cl is 300 mgm. per 100 cc. Column 2 gives similar ratios taken from Corper (3) for the distribution of CNS after intravenous injection. The figures in Column 3 were calculated from the data of the first experiment of Denis and Leche (9). In all cases the animals used for analysis were killed without exsanguination. The three sequences, considering their varied sources, are sufficiently similar to warrant the assumption that Cl, CNS and  $\text{SO}_4$  are distributed through approximately the same volume of fluid. Experiments of Greenwald (13) indicate that intravenously injected inorganic phosphate distributes itself like sulfate. A recent experiment in this department indicates that magnesium, injected as magnesium sulfate, behaves in the same manner.

The experiments in Table IV were devised to test in another manner the distribution of sulfate and its accessibility to the cells. For this purpose rather large amounts of hypertonic sodium sulfate were injected intravenously. Blood was withdrawn before the injections and shortly after the injections which took from 25 to 45 minutes. Time was not, therefore, allowed for complete distribution of the sulfate. Urine was voided before the injection and collected at the time of the second venous puncture in order that correction could be made for injected salt lost by excretion. If the hypotheses which have been expressed are correct, injection of such a hypertonic

solution should cause water to be transferred from the cells to the interstitial fluids in which the injected sodium sulfate should remain, while endogenous sodium and chloride should be diluted by the water injected and that derived from the cells. Thiocyanate, which was administered the preceding night to provide a further criterion of volume changes, should be diluted to an equivalent extent.

Attention may be turned first to the changes in the blood, with Experiment 14 as an example. If it is assumed that the total circulating hemo-

globin did not change, the blood volume, from the dilution of hemoglobin (oxygen capacity), expanded  $100\left(\frac{18.1-16.7}{16.7}\right)=8$  per cent. Since the ratio, oxygen capacity: cell volume, rose from  $18.1/39.4=0.459$  to  $16.7/34.7=0.481$ , the original cells must have shrunk  $100\left(\frac{0.481-0.459}{0.481}\right)=4.6$  per cent. As cells contain only 70 per cent of water by volume, this means, in actual point of fact, that the cells yielded 6.6 per cent of their water to the serum. The serum, meanwhile, esti-

TABLE IV  
*The effect of large intravenous injections of sodium sulfate*

Time from beginning of injection	Serum					Blood		Volume of distribution of		Approximate volume	Urine		
	SO <sub>4</sub>	Cl	Total CO <sub>2</sub>	Total base	Proteins	O <sub>2</sub> capacity	Cells	SO <sub>4</sub>	CNS		SO <sub>4</sub>	Cl	Na
minutes	m.eq. per liter	m.eq. per liter	m.eq. per liter	m.eq. per liter	per cent	volumes per cent	volumes per cent	liters	liters	cc.	m.eq.	m.eq.	m.eq.
Experiment 9, Normal female, body weight 58 kgm., 85.2 m.eq. injected in 12 minutes													
0	0.9	97.6	26.7		7.3								
17	9.1	90.4	25.7		7.1			9.1					
Experiment 10, Normal male, body weight 86 kgm., 93 m.eq. injected in 10 minutes													
0	1.0	97.5	28.5										
45	5.5	92.4	27.4					13.5		140	33.0	10.7	30.9
105										80	19.4	5.3	16.5
Experiment 11, Female, mild diabetic, body weight 70 kgm., 270 m.eq. injected in 45 minutes													
0	0.6	96.0	28.0		6.9								
45	16.2	88.8	28.0		6.0			13.4		200	62.2	6.0	63.8
105	9.4	93.6	27.6		7.1			15.3		200	74.5	0.7	67.8
Experiment 12, Male, mild diabetic, body weight 71 kgm., 270 m.eq. injected in 25 minutes													
0	(0.5)*	96.0	29.0	135.6†	6.7								
30	14.5	96.2	27.4	144.5†	5.5			16.5	20.1		40.3	18.0	55.0
130									20.5		95.5	8.0	90.0
									19.9				
Experiment 13, Normal male, body weight 59 kgm., 258 m.eq. injected in 30 minutes													
0	(0.5)*	98.5	26.0	148.8	6.4	20.5	47.5		16.2				
40	15.3	93.1	25.2	154.5	5.6	18.6	41.8	12.9	17.1	245	67.4	13.0	63.0
Experiment 14, Normal female, body weight 55 kgm., 266 m.eq. injected in 45 minutes													
0	(0.5)*	104.9	26.2	148.6	7.3	18.1	39.4		13.1				
50	20.3	99.2	22.5	155.9	6.5	16.7	34.7	9.6	14.1	260	76.9	9.0	80.0

\* Assumed figure.

† Sodium only.

mated from the change of serum proteins, expanded  $\frac{7.3-6.5}{6.5} = 12$  per cent. From cell volume and oxygen capacity measurements its expansion can be calculated as follows: 100 cc. of whole blood, which contained 39.4 per cent of cells, became, after the injection, 100 cc.  $\times 18.1/16.7 = 108$  cc., containing 34.7 per cent of cells, or 108 cc.  $\times 34.7/100 = 37.5$  cc.; therefore the change in serum volume was from 100 cc. — 39.4 cc. = 60.6 cc. to 108 cc. — 37.5 cc. = 70.5 cc., which is an increase of 16 per cent. The agreement by the two methods is highly satisfactory considering the double assumption that neither the cells nor the protein in the circulation changed during the experiment. The concentration of base in the serum, if this may be taken as a measure of osmotic pressure, rose from 148.6 to 155.9, or about 5 per cent. The results, as far as the blood itself is concerned, then, are quite consonant with theory. The hypertonic solution withdrew, perhaps only temporarily, some interstitial fluid into the blood stream and at the same time stole enough from the cells to reestablish osmotic equilibrium without necessitating any transfer of base. The results in Experiment 13 are quite comparable. The blood volume increased 10 per cent; the cells contracted 3 per cent, cell water 4 per cent; serum volume expanded 14 per cent (from proteins) or 22 per cent (from cell volume and oxygen capacity); the concentration of base in serum rose 4 per cent.

Calculations of the exchanges of fluids and solutes in the other body fluids are more complicated. In Experiment 14, from the change of the concentration of chloride in serum the interstitial fluids would seem to have expanded  $100\left(\frac{104.9-99.2}{99.2}\right) = 5.7$  per cent (if the loss of 9 m.eq. of Cl in the urine is neglected). If sodium were diluted to the same extent, the concentration of endogenous sodium in the serum at the end of the experiment should have been  $148.6/105.7 = 140.6$  m.eq. The difference between this and the 155.9 m.eq. of sodium actually found amounts to 15.3 m.eq., which agrees fairly well with the increment of sulfate,  $20.3 - 0.5 = 19.8$  m.eq. The changes can be evaluated in a slightly different manner. Since the amounts of

SO<sub>4</sub> and of Na excreted are so nearly identical it may be assumed that exogenous increments of Na and SO<sub>4</sub> are the same. Because endogenous SO<sub>4</sub> is negligibly small, the magnitude of these increments can be estimated from SO<sub>4</sub> as 19.8 m.eq. In this case the final endogenous Na must have been  $155.9 - 19.8 = 136.1$  m.eq. and dilution, estimated from Na,  $100\left(\frac{148.6-136.1}{136.1}\right) = 9.2$  per cent. From the serum SO<sub>4</sub> and the quantity of SO<sub>4</sub> given, corrected for excreted SO<sub>4</sub> and endogenous SO<sub>4</sub>, the estimated volume of the interstitial fluids at the end of the injection is  $\frac{266 - (76.9 - 0.8)}{20.3 - 0.5} = 9.6$  kgm., which would

mean that before the injection it was, by the calculations above, from Cl  $9.6/105.7 = 9.1$ , from SO<sub>4</sub> and Na  $9.6/109.1 = 8.9$  kgm. This would mean a gain of 0.5 to 0.8 kgm. of water. The fluid injected minus the urine excreted amounted to  $500 - 260 = 240$  cc. If extrarenal losses of water are neglected, then, by this method of calculation at least  $(0.5 \text{ to } 0.8) - 0.2 = 0.3$  to 0.6 kgm. of water must have been derived from the cellular fluids. The interstitial fluid volume estimated from thiocyanate is, in this instance, far larger than that calculated from the distribution of sulfate and of a more plausible magnitude. The explanation for the difference probably lies in the fact that time was not given for the complete diffusion of sulfate, while thiocyanate, which had been administered the night before, was evenly distributed through the body fluids. Thiocyanate, therefore, should be a more suitable criterion of both the original volume and the change of volume of the interstitial fluids. From thiocyanate the interstitial fluids should have gained  $1.0 - 0.2 = 0.8$  kgm. of water from the cells, becoming diluted to the extent of about 8 per cent.

If it be assumed that the body of the subject, who weighed 55 kgm., contained altogether 70 per cent by weight of water, the total body water was 38.5 kgm., and the cellular water (from KCNS)  $38.5 - 13.1 = 25.4$  kgm. Of this,  $\frac{100 \times 0.8}{25.4}$  or about 3 per cent was transferred to the extracellular fluids.

If osmotic equilibrium between cells and interstitial fluid was maintained in spite of the salt

solution should cause water to be transferred from the cells to the interstitial fluids in which the injected sodium sulfate should remain, while endogenous sodium and chloride should be diluted by the water injected and that derived from the cells. Thiocyanate, which was administered the preceding night to provide a further criterion of volume changes, should be diluted to an equivalent extent.

Attention may be turned first to the changes in the blood, with Experiment 14 as an example. If it is assumed that the total circulating hemo-

globin did not change, the blood volume, from the dilution of hemoglobin (oxygen capacity), expanded  $100 \left( \frac{18.1 - 16.7}{16.7} \right) = 8$  per cent. Since the ratio, oxygen capacity: cell volume, rose from  $18.1/39.4 = 0.459$  to  $16.7/34.7 = 0.481$ , the original cells must have shrunk  $100 \left( \frac{0.481 - 0.459}{0.481} \right) = 4.6$  per cent. As cells contain only 70 per cent of water by volume, this means, in actual point of fact, that the cells yielded 6.6 per cent of their water to the serum. The serum, meanwhile, esti-

TABLE IV  
*The effect of large intravenous injections of sodium sulfate*

Time from beginning of injection	Serum					Blood		Volume of distribution of		Urine				
	SO <sub>4</sub>	Cl	Total CO <sub>2</sub>	Total base	Proteins	O <sub>2</sub> capacity	Cells	SO <sub>4</sub>	CNS	Approximate volume	SO <sub>4</sub>	Cl	Na	
minutes	m.eq. per liter	m.eq. per liter	m.eq. per liter	m.eq. per liter	per cent	volumes per cent	volumes per cent	liters	liters	cc.	m.eq.	m.eq.	m.eq.	
Experiment 9, Normal female, body weight 58 kgm., 85.2 m.eq. injected in 12 minutes														
0	0.9	97.6	26.7		7.3									
17	9.1	90.4	25.7		7.1			9.1						
Experiment 10, Normal male, body weight 86 kgm., 93 m.eq. injected in 10 minutes														
0	1.0	97.5	28.5											
45	5.5	92.4	27.4					13.5		140	33.0	10.7	30.9	
105										80	19.4	5.3	16.5	
Experiment 11, Female, mild diabetic, body weight 70 kgm., 270 m.eq. injected in 45 minutes														
0	0.6	96.0	28.0		6.9									
45	16.2	88.8	28.0		6.0			13.4		200	62.2	6.0	63.8	
105	9.4	93.6	27.6		7.1			15.3		200	74.5	0.7	67.8	
Experiment 12, Male, mild diabetic, body weight 71 kgm., 270 m.eq. injected in 25 minutes														
0	(0.5)*	96.0	29.0	135.6†	6.7									
30	14.5	96.2	27.4	144.5†	5.5			16.5	20.1		40.3	18.0	55.0	
130									20.5		95.5	8.0	90.0	
									19.9					
Experiment 13, Normal male, body weight 59 kgm., 258 m.eq. injected in 30 minutes														
0	(0.5)*	98.5	26.0	148.8	6.4	20.5	47.5		16.2					
40	15.3	93.1	25.2	154.5	5.6	18.6	41.8	12.9	17.1	245	67.4	13.0	63.0	
Experiment 14, Normal female, body weight 55 kgm., 266 m.eq. injected in 45 minutes														
0	(0.5)*	104.9	26.2	148.6	7.3	18.1	39.4		13.1					
50	20.3	99.2	22.5	155.9	6.5	16.7	34.7	9.6	14.1	260	76.9	9.0	80.0	

\* Assumed figure.

† Sodium only.

mated from the change of serum proteins, expanded  $\frac{7.3 - 6.5}{6.5} = 12$  per cent. From cell volume and oxygen capacity measurements its expansion can be calculated as follows: 100 cc. of whole blood, which contained 39.4 per cent of cells, became, after the injection, 100 cc.  $\times 18.1/16.7 = 108$  cc., containing 34.7 per cent of cells, or 108 cc.  $\times 34.7/100 = 37.5$  cc.; therefore the change in serum volume was from 100 cc. — 39.4 cc. = 60.6 cc. to 108 cc. — 37.5 cc. = 70.5 cc., which is an increase of 16 per cent. The agreement by the two methods is highly satisfactory considering the double assumption that neither the cells nor the protein in the circulation changed during the experiment. The concentration of base in the serum, if this may be taken as a measure of osmotic pressure, rose from 148.6 to 155.9, or about 5 per cent. The results, as far as the blood itself is concerned, then, are quite consonant with theory. The hypertonic solution withdrew, perhaps only temporarily, some interstitial fluid into the blood stream and at the same time stole enough from the cells to reestablish osmotic equilibrium without necessitating any transfer of base. The results in Experiment 13 are quite comparable. The blood volume increased 10 per cent; the cells contracted 3 per cent, cell water 4 per cent; serum volume expanded 14 per cent (from proteins) or 22 per cent (from cell volume and oxygen capacity); the concentration of base in serum rose 4 per cent.

Calculations of the exchanges of fluids and solutes in the other body fluids are more complicated. In Experiment 14, from the change of the concentration of chloride in serum the interstitial fluids would seem to have expanded  $100 \left( \frac{104.9 - 99.2}{99.2} \right) = 5.7$  per cent (if the loss of 9 m.eq. of Cl in the urine is neglected). If sodium were diluted to the same extent, the concentration of endogenous sodium in the serum at the end of the experiment should have been  $148.6/105.7 = 140.6$  m.eq. The difference between this and the 155.9 m.eq. of sodium actually found amounts to 15.3 m.eq., which agrees fairly well with the increment of sulfate,  $20.3 - 0.5 = 19.8$  m.eq. The changes can be evaluated in a slightly different manner. Since the amounts of

SO<sub>4</sub> and of Na excreted are so nearly identical it may be assumed that exogenous increments of Na and SO<sub>4</sub> are the same. Because endogenous SO<sub>4</sub> is negligibly small, the magnitude of these increments can be estimated from SO<sub>4</sub> as 19.8 m.eq. In this case the final endogenous Na must have been  $155.9 - 19.8 = 136.1$  m.eq. and dilution, estimated from Na,  $100 \left( \frac{148.6 - 136.1}{136.1} \right) = 9.2$  per cent. From the serum SO<sub>4</sub> and the quantity of SO<sub>4</sub> given, corrected for excreted SO<sub>4</sub> and endogenous SO<sub>4</sub>, the estimated volume of the interstitial fluids at the end of the injection is  $\frac{266 - (76.9 - 0.8)}{20.3 - 0.5} = 9.6$  kgm., which would

mean that before the injection it was, by the calculations above, from Cl  $9.6/105.7 = 9.1$ , from SO<sub>4</sub> and Na  $9.6/109.1 = 8.9$  kgm. This would mean a gain of 0.5 to 0.8 kgm. of water. The fluid injected minus the urine excreted amounted to  $500 - 260 = 240$  cc. If extrarenal losses of water are neglected, then, by this method of calculation at least  $(0.5 \text{ to } 0.8) - 0.2 = 0.3 \text{ to } 0.6$  kgm. of water must have been derived from the cellular fluids. The interstitial fluid volume estimated from thiocyanate is, in this instance, far larger than that calculated from the distribution of sulfate and of a more plausible magnitude. The explanation for the difference probably lies in the fact that time was not given for the complete diffusion of sulfate, while thiocyanate, which had been administered the night before, was evenly distributed through the body fluids. Thiocyanate, therefore, should be a more suitable criterion of both the original volume and the change of volume of the interstitial fluids. From thiocyanate the interstitial fluids should have gained  $1.0 - 0.2 = 0.8$  kgm. of water from the cells, becoming diluted to the extent of about 8 per cent.

If it be assumed that the body of the subject, who weighed 55 kgm., contained altogether 70 per cent by weight of water, the total body water was 38.5 kgm., and the cellular water (from KCNS)  $38.5 - 13.1 = 25.4$  kgm. Of this,  $\frac{100 \times 0.8}{25.4}$

or about 3 per cent was transferred to the extracellular fluids.

If osmotic equilibrium between cells and interstitial fluid was maintained in spite of the salt

which was injected and if it may be assumed that with the exception of the inorganic salts and proteins there are no osmotically important solutes to which the cellular membranes are not permeable, it follows: (1) that the effective osmotic pressure in intracellular and extracellular fluids is related to the concentration of salt in these fluids and (2) that a change in the concentration of salt in one medium must be adjusted by an exchange of water or salt such that at equilibrium the concentrations of salt in the two media will again be equal.

if it was 0.24 kgm.,  $160.4W + 182 = 166.8 (W + 0.24)$  and  $W = 22.2$  kgm. Both these figures are far below the theoretical estimate,  $0.7 \times \text{body weight} = 38.5$  kgm. This is to be expected, however, if the distribution of  $\text{SO}_4$  has not been completed.

The calculations for Experiment 14 have been given in full to illustrate the procedures employed. The results of similar calculations for Experiments 13 and 12 are presented in Table V. The results of Experiment 13 are in all respects com-

TABLE V  
*Approximate calculations of exchange of water in the body after intravenous injection of sodium sulfate*

	Experiment		
	12	13	14
Expansion of interstitial fluid from $[\text{Cl}]$ , per cent. ....	0	5.8	5.7
Expansion of interstitial fluid from $[\text{CNS}]$ , per cent. ....	2.0	5.5	7.6
Expansion of interstitial fluid from endogenous $[\text{Na}]$ , per cent. ....	3.9	6.5	9.2
Final endogenous $[\text{Na}]$ from $[\text{Cl}]$ , m.eq. ....	135.9	140.7	140.6
Final endogenous $[\text{Na}]$ from $[\text{CNS}]$ , m.eq. ....	133.0	141.0	138.2
Final endogenous $[\text{Na}]$ from $[\text{SO}_4]$ , m.eq. ....	130.5	139.7	136.1
Total volume of body fluids, kgm.: as $(0.7 \times \text{body weight})$ .....	49.7	41.3	38.5
as $W = \frac{B_e - [B]_s''E}{[B]_s - [B]_s'}$ if $E = 0$ .....	28.3†	32.9†	28.4
as $W = \frac{B_e - [B]_s''E}{[B]_s - [B]_s'}$ if $E = 0.24$ .....			22.2
Increment of interstitial fluid: from cells, kgm. ....	<0.4†	0.7	0.8
as per cent of cell water * .....	<1.4†	2.8	3.1

\* Cell water =  $(0.7 \text{ body weight}) - (\text{interstitial fluid from CNS})$ .

† In this experiment  $E$  is derived from the change of body weight (0.22 kgm.) which was measured.  $K$  excretion was 3.9 m.eq.

‡ Urine volume was not measured.

If total base of serum may serve as a rough approximation of the osmolar concentration of salt,

$$[B]_s'W + B_e = [B]_s''(W + E)$$

in which  $[B]_s'$ ,  $[B]_s''$  and  $B_e$  represent respectively the concentrations of base in serum before and after the injection and the increment of exogenous base,  $W$  the initial body water and  $E$  the increment of exogenous water (20). In Experiment 14,  $E$  was not measured, but its limits can be approximately defined. It can hardly have exceeded 0.24 kgm., the difference between the fluid injected and the urine excreted. If it was 0,  $160.4W^2 + 182^2 = 166.8W$ , and  $W = 28.4$  kgm.;

$^2 [B]_s'$  and  $[B]_s''$  are here expressed in m.eq. per liter of water, estimated by the equation, serum water = serum volume  $(100 - \text{protein concentration})$ .  $B_e = \text{Na}e + \text{K}e$ , the potassium excreted amounting to 3.7 m.eq.

parable to those of Experiment 14. Except that chloride remained unaltered in Experiment 12, the changes in the serum are similar to those in the other experiments. The calculated changes in the volumes of the various body fluids are, however, far smaller. This may be due in part to the larger size of the subject, but can probably be attributed chiefly to the fact that equilibrium has been less perfectly established. In this experiment the fluid was injected more rapidly than in the other two and the second blood sample was withdrawn earlier, only 30 minutes after the beginning of the injection. This view finds support in the fact that serum proteins were more diluted (22 per cent) in Experiment 12 than in the other two although the volume of the blood, judging from the size of the subject, must have been

larger. It is unfortunate that neither oxygen capacity nor cell volume was measured.

The data can be tested in a somewhat different manner. If the volume of interstitial fluid,  $F$ , from CNS is correct, the total amount of Cl in the body before the injection should be (assuming Cl distribution to be approximately equal in serum and extravascular spaces)  $[Cl]_s' F'$ , and at the end  $[Cl]_s'' F''$ , and

$$[Cl]_s' F' = [Cl]_s'' F'' + \Delta Cl,$$

$\Delta Cl$  being the balance of Cl during the experiment. This permits the calculation of  $[Cl]_s''$ . The same method may be applied to the analysis of the figures for base and for  $SO_4$ . The results of such calculations give in Experiment 14:  $[Cl]_s''$  calculated 96.8, found 99.2;  $[B]_s''$  calculated 151.3, found 155.9;  $[SO_4]_s''$  calculated 13.9, found 20.3; for Experiment 13:  $[Cl]_s''$  calculated 92.6, found 93.1;  $[B]_s''$  calculated 152.4, found 154.5;  $[SO_4]_s''$  calculated 11.7, found 15.3; for Experiment 12:  $[Cl]_s''$  calculated, 93.3, found 96.2;  $[B]_s''$  calculated, 143.5, found 144.5;  $[SO_4]_s''$  calculated 11.8, found 14.5. In all experiments  $SO_4$  gives the worst agreement, which might be expected if equilibrium had not been attained, since  $[Cl]_s$  is affected only by dilution,  $[SO_4]_s$  only by diffusion, and  $[Na]_s$  by both.

Experiments 9, 10 and 11 are incomplete in so many respects that they can not be treated in the same detail. As far as they go, they agree with the more complete experiments.

When the interchanges in the components of the blood are compared with the shifts of water between tissues and interstitial fluids, the former are found to be far more satisfactory. In Experiment 14 the blood cells gave up 7 per cent of their water when the concentration of base in the serum rose 5 per cent; in Experiment 13 the agreement is still more exact, 4 per cent of water issuing from the cells in response to a 4 per cent increment of base. For the same increase of base the estimated loss of water from the tissue cells are only 4 per cent in Experiment 14, 3 per cent in Experiment 13. Again, however, it must be emphasized that equilibrium with the tissue cells was probably not established. Equilibrium within the blood must have been attained, because adjustments which were incomplete when the blood

was withdrawn, were free to proceed to equilibrium *in vitro*.

On the whole the data are compatible with the hypothesis that Cl, Na,  $SO_4$  and CNS are effectively excluded from the cells of the body, and that when the concentration of sodium in the body is increased, uniformity of osmotic pressure between cells and interstitial fluids is restored by transfer of water from the former to the latter.

#### COMMENT

Denis (7) claimed that the kidney selectively retained the  $SO_4$  ion when  $Na_2SO_4$  was injected. This opinion was based on analyses of serum only. It is clear from the experiments reported above that Na and  $SO_4$  are excreted at the same speed, a thing which Greenwald's (13) experiments on dogs had already shown. Serum determinations alone will induce to error if one forgets that a dilution of serum may mask completely an increase of serum Na, and leave an increase of  $SO_4$  practically unaffected. For example, if, to 1000 cc. of serum, containing initially 130 m.eq. of Na and 1 m.eq. of  $SO_4$ , is added 13 m.eq. of  $Na_2SO_4$  in 100 cc. of water, the Na concentration remains  $\frac{130 + 13 \text{ m.eq.}}{1000 + 100 \text{ cc.}} = 130$  m.eq. per liter, whereas the  $SO_4$  concentration becomes  $\frac{1 + 13 \text{ m.eq.}}{1000 + 100 \text{ cc.}} = 12.7$  m.eq. per liter.

Neglect of this consideration in Experiments 13 and 14, in which the apparent increment of  $SO_4$  was almost three times that of total base, would lead to a completely erroneous interpretation. When a substance in hypertonic solution is introduced into the parenteral spaces, the osmotic disturbance it creates tends to be compensated immediately: if the substance can diffuse evenly into the total body fluids, including cell water, a new equilibrium will be reached in this manner; if, as for  $Na_2SO_4$ , its diffusion is limited to the extracellular fluids, a new equilibrium will be reached only through an outpour of water from the cells. When Denis and von Meysenbug (10) thought that NaCl and  $Na_2SO_4$  injections in dogs had an acidifying effect because they observed a sharp drop of  $CO_2$  and a slight drop of pH, the solutions they used were so large and concentrated that the apparent acidosis was probably mostly



an effect of dilution. Similar, but lesser reductions of  $\text{CO}_2$  are seen in the experiments of Table IV. As Peters (24) has pointed out, if it *seems* that when sodium is injected it diffuses into the total body water, it is because the organism tends to distribute the increase in osmolar concentration equally in all body fluids by increasing the water content of the extracellular fluid which contains the sodium, and decreasing correspondingly the water content of the cells.

A word should be added about the position of the  $\text{SO}_4$  and the CNS ions in the lyotropic series (12, 28). It is known that  $\text{SO}_4$  permeates membranes most slowly, CNS most rapidly, so that one could expect to observe a considerable difference in the speed of diffusion of these ions in the body. Actually, the difference, though demonstrable, is not considerable.

On the whole, the impression gained is that sodium sulfate parenterally introduced behaves in a fashion which is probably common to most electrolytes, diffusing into the interstitial fluid only and creating thus osmotic disturbances which have to be readjusted through transfers of water. At the same time it is excreted by the kidney at a rate proportional to its concentration in the body.

#### SUMMARY

The effect of sodium sulfate intravenously injected in hypertonic solutions was tested in man. Subjects were healthy men and women and patients with chronic nephritis. Doses injected ranged from 1.3 to 20 grams, and were in most cases accompanied by sodium thiocyanate. In some cases, potassium thiocyanate was given perorally twelve hours before.

A few preliminary data are given on the distribution of endogenous sulfate between serum and transudates.

It was found that the sodium ion and the sulfate ion were excreted at the same rate, and that the amount of salt injected could probably be recovered totally in urine. The rate of sulfate excretion appeared to be simply proportional to the concentration of sulfate in serum.

The figures obtained for the diffusion of sulfate suggest that, like thiocyanate, it is distributed only in the interstitial fluid. The larger doses of sulfate were found to cause usually a dilution of

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# A COMPARISON OF THE ELECTROPHORETIC MOBILITIES AND SEDIMENTATION VELOCITIES OF RED CELLS FROM NORMAL AND PREGNANT HUMAN SUBJECTS<sup>1</sup>

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It has long been known that the extent to which red cells aggregate (as measured by sedimentation velocity) in their own plasma varies greatly with the species. Also, within one species (human) the degree of aggregation varies with the physiological or pathological state of the individual, being greatly increased above the normal during pregnancy and in many febrile and neoplastic disorders. It has recently been shown (1) that the main cause of species variations in sedimentation velocity is a difference in the red cells themselves, rather than in the plasma, since cells from various species sink at very different rates in a common medium such as 1 per cent gelatin. This species difference is correlated, 1, with the electrophoretic mobility of the red cells, the cells which settle most rapidly having the highest mobility at pH 7.4, 2, with the isoelectric point, the cells with highest sedimentation velocity having the lowest isoelectric point, and, 3, presumably with the chemical composition of the red cell surface, those cells sinking most rapidly which have the highest proportion of lipid to protein with a resultant higher interfacial energy.

The increased rate of sedimentation accompanying pregnancy and pathological conditions in man, on the other hand, has long been assigned to an increase in the globulin and particularly in the fibrinogen fraction of the plasma. That an increase in plasma globulin takes place during these conditions has been repeatedly demonstrated; that such an increase in globulin concentration will directly affect the sedimentation velocity of the red cells is equally certain (2). The possibility of a difference in the cells of slowly and rapidly settling human blood comparable to that observed in different species has

not, however, been excluded. Fåhræus (2) observed the sedimentation of cells from normal males, in plasma from pregnant individuals, and *vice versa* and concluded that differences in the rate of aggregation and sinking are chiefly dependent on the properties of the plasma. The corpuscles from pregnant individuals, however, always sank somewhat more rapidly in a given plasma than the cells from males.

In order to avoid any group specific agglutination we have compared the sedimentation velocities of cells from normal and pregnant individuals in a common artificial medium, namely, 1 per cent gelatin, as well as in their own plasmas. Measurements of the electrophoretic mobility of cells from normal and pregnant people in a buffer medium at pH 7.4 were also made in order to detect, if possible, differences in the chemical make-up of the cells from such sources (1).

## EXPERIMENTAL

Fresh oxalated blood, not more than 3 hours old, was used. All the blood samples for a single experiment were centrifuged at the same time and the sedimentation velocities measured in the same sample of 1 per cent gelatin. Determinations of electrophoresis were made in a cylindrical cell of a modified Mattson type. Observations were at a level of 0.147 cell diameter below the cell roof, at which level there is no electroosmotic movement. The visual axis was the vertical diameter of the cell. A field strength of 9.1 volts per centimeter was used; five observations of red cell movement for each direction of current were made, and the average velocity computed. The cells were suspended in M/50 phosphate buffer of pH 7.4 plus 0.3 per cent NaCl plus 6 per cent sucrose, the latter being substituted for part of the salt to cut down current density.

The 1 per cent gelatin used in the sedimenta-

<sup>1</sup> Aided by a grant by the Rockefeller Foundation to Washington University for research in science.

tion experiments was made up in M/50 phosphate buffer at pH 7.4 plus 0.9 per cent NaCl. The cells were centrifuged for fifteen minutes at 2000 r.p.m. and 2 volumes of the gelatin solution or autogenous plasma added to 1 of packed cells. The thoroughly mixed cell suspensions were sucked up into glass tubes of 3.5 mm. bore and 35 cm. long. The tubes bore short segments of rubber tubing on their lower ends which, after filling, were closed with spring clips. The number of millimeters which the cells sank in a specified time was measured. The precautions of maintaining a constant ratio of cells to volume of the suspension medium and of having the tube sufficiently long that the rate of sedimentation is not slowed by a beginning packing are essential to quantitative comparisons. The former precaution is practically always and the latter often disregarded in clinical work.

The results are shown in Table I. Only the measurements within a given experiment are comparable, since on different days the solutions used and conditions such as temperature might vary. It is evident that no measurable difference exists between the electrophoretic mobilities of cells from normal and pregnant individuals. This confirms the work of Abramson (3). Fåhræus' (4) original statement that cells from male subjects show a greater mobility than those from pregnant subjects was admittedly based upon non-quantitative observations and was apparently retracted by him three years later (2), although this point has frequently escaped notice in the subsequent literature.

It is also evident that, whereas cells from pregnant people sink much more rapidly (from 3 to 25 times) than cells from non-pregnant individuals in their own respective plasmas, there is little or no difference between the sinking velocities of the two types of cells in 1 per cent gelatin. In Experiment 1 there is no correlation between the condition of the subject from which the cells came and the sedimentation velocity of the cells in gelatin. In Experiment 2 the slight (40 to 50 per cent) increase in sedimentation of the pregnant over the normal cells in gelatin is probably to be ascribed to the effect of the globulin-rich plasma remaining in the packed cell mass after centrifugation. This conclusion is supported by

TABLE I

*Sedimentation velocity and electrophoretic mobility of cells from pregnant and non-pregnant human subjects*

Condition of subject	Electrophoretic mobility	Sinking in 1 per cent gelatin	Sinking in autogenous plasma
	<i>micra per second per volt per cm.</i>	<i>mm. in 50 minutes</i>	<i>mm. in 60 minutes</i>
Experiment 1			
Normal male.....	1.22	87	3
Normal female.....	1.25	74	8
Normal male.....	1.22	103	3
At term.....	1.23	120	76
At term.....	1.24	99	43
14 hours postpartum.....	1.25	55	17
Experiment 2		<i>mm. in 50 minutes</i>	<i>mm. in 50 minutes</i>
Normal female.....	1.39	81	4
Normal female.....	1.42	81	5
Normal female.....	1.40	70	6
At term.....	1.46	128	60
At term.....	1.39	117	30
At term.....	1.37	111	49
Experiment 3		<i>mm. in 50 minutes</i>	<i>mm. in 50 minutes</i>
Normal female.....	1.24	39	8
Normal female.....	1.27	49	1
Normal female.....	1.25	35	9
At term.....	1.27	40	55
At term.....	1.24	42	31
5 hours postpartum.....	1.28	31	28

the findings of Experiment 3, in which the cells previously to being suspended in 1 per cent gelatin were washed with 0.9 per cent sodium chloride solution to free them of plasma. It is seen that the cells from the pregnant subjects sank at the same rate in 1 per cent gelatin as did those from the normal non-pregnant ones. Differences observed are not sufficiently great to warrant the conclusion that a chemical difference, comparable to that demonstrated for different species, exists in the cells of normal as compared with those of pregnant human subjects.

#### SUMMARY

Whereas red cells from pregnant human subjects sink in their own plasma from 3 to 25 times more rapidly than do cells from normal people in their own plasma, no consistent difference is found in the respective sedimentation velocities

in a common medium, 1 per cent gelatin. Also, no measurable difference exists in the electrophoretic mobility of cells from normal as compared with that of cells from pregnant individuals in a common buffer medium. It is concluded that the increased sedimentation velocity of cells in blood from pregnant people is entirely due to changes that occur in the plasma during pregnancy.

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# THE EFFECT OF THE TOXEMIAS OF PREGNANCY ON RENAL FUNCTION

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In a previous contribution (1) it was shown that there is a wider range of normal values for renal function tests in normal women in the last trimester of pregnancy than in normal non-pregnant individuals. The blood urea clearance (2) was shown to vary between 60 and 118 per cent of normal. The total protein content of the urine was within normal limits. The urinary sediment count of Addis (3) showed the casts to vary from 0 to 10,000, the red blood cells to range from 47,000 to 1,900,000 and the white blood and epithelial cells to vary from 25,000 to 6,000,000. These are the values which must be used for comparison when interpreting the results of renal function tests during the acute phase of the toxemias of pregnancy. When the patient has returned to her normal non-pregnant level, some three months postpartum, comparison can be made with the usual normal values established by Van Slyke and Addis.

The above tests with the routine procedures as previously outlined (1) were made on each of thirty-three patients on three occasions; during the acute phase of the toxemia prior to delivery, in the early puerperium and three or more months postpartum. This method of study was followed in order that the course of renal function could be studied with the possible end in view of an earlier and better prognosis and perhaps a more simple classification of the toxemias of pregnancy. The cases studied have been divided into three groups; (I) preeclampsia including all types from the mild to the more severe; (II) eclampsia; and (III) nephritis complicating pregnancy. The results are shown in Tables I, II and III respectively. The cases in Tables I and II are subdivided into two groups, namely, those with no renal damage and those with possible renal damage.

## RESULTS

### *Preeclampsia (Table I)*

This group includes twenty cases of preeclampsia varying from the milder forms with elevated blood pressure and albuminuria to those verging on eclampsia. They are about equally divided between the two subgroups. There was no history of nephritis in any of the cases. In nine of the twenty cases there was a history of a previous preeclampsia. The cases with a history of a previous toxemia are about equally divided between the two subgroups and are identified by an asterisk under the case number. In two of the cases with previous preeclampsia the recent clinical course was sufficiently severe and the renal function sufficiently impaired to warrant termination of the pregnancy. Both of these cases (52767 and 59342) have sustained definite renal injury.

Considering the renal function of this group as a whole it will be noted that during the acute phase the blood urea clearance is for the most part normal. In three cases it was below 60 per cent of normal, the lower normal limit during the last trimester of pregnancy. During the puerperium little change in the blood urea clearance was observed. In the period three or more months postpartum, however, there were four of the nine cases with possible renal damage in which the blood urea clearance was below the lower limits established by Van Slyke. The values obtained were sufficiently low to indicate renal injury.

The degree of proteinuria (4) is of no marked diagnostic or prognostic significance during the acute phase of the toxemia or in the early puerperium. When it is observed in abnormal amounts in the period three months postpartum as it was in three of the nine cases with possible renal damage it serves to indicate renal injury.

The Addis sediment count during the acute phase of the toxemia showed an abnormally high number of casts, erythrocytes, leukocytes and epi-

thelial cells. The numbers were greatly reduced in the early puerperium but still remained high enough to have pathological significance. During the acute phase and in the early puerperium there was no correlation between the Addis count and the blood urea clearance. In the period three months postpartum the Addis count returned to the normal limits as established by Addis in all cases in the subgroup with no renal damage. In the subgroup with possible renal damage the cast

count remained abnormally high in all but two cases and in these two cases the blood urea clearance was below the normal limits established by Van Slyke. In two of the cases in this group the erythrocytes and leukocytes of the urine were above normal.

The results of these observations reveal that of the cases of preeclampsia about half do and half do not show evidence of renal injury three months postpartum. It will be of interest to fol-

TABLE I  
*Renal function tests in preeclampsia*

Case number	Age	Para	Days †	Blood pressure	Urea clearance ‡		Protein-uria	Addis count			Remarks
					1st hour	2nd hour		Casts	Red blood cells	White blood cells	
					per cent of normal	per cent of normal		thousands per 12 hours	millions per 12 hours	millions per 12 hours	
	years			mm. Hg			grams per 12 hours				
NO RENAL DAMAGE											
64985*	27	4	110 PP 575 PP 130 AP 11 PP	140/110 130/95 130/100	146 M 225 M 93 M	207 M 155 M 93 M	.04 .18 Trace	11.6 0 7	.14 .27 .64	.18 .54 2.3	Labor induced at term
88160	24	0	12 PP 120 PP	130/80 130/80	93 M 80 M	100 M 70 M	.04 .18	13.8 0	.50 .08	3.6¶ 1.0	
68626	21	0	2 AP 9 PP 100 PP	150/100 130/80 120/90	55 S 80 S 65 M	36 S 57 S 84 M	.75 .8 .02	1§ 49 7.1	.04 .02 .02	.02 .05 .03	
73554	27	0	31 AP 19 PP 240 PP 180 AP	120/70 140/90 120/60	74 M 87 M 105 M	67 M 63 M 105 M	1.03 .04 .18	7 18 7	.26 1.15 .13	4.5 5.7 .39	Pregnant
29606*	29	3	3 AP 320 PP	140/80 122/80	132 S 200 M	104 S 100 M	0 .15	§ 0	§ .27	§ .45	
27843*	27	0	34 AP 11 PP 185 PP	140/90 140/98 134/80	145 M 80 M 84 M	145 M 84 M 66 M	.3 .07 .18	18 17 7	.14 .31 .13	.76 .45 .15	
72336	22	0	1 AP 9 PP 255 PP	150/110 120/80 128/62	52 S 73 S 82 M	68 S 89 M 77 M	1.32 1.04 .18	150 20 5.3	2.8 2.1 30.4	3.3 21.8¶ .29	
66730	19	0	3 AP 97 PP	150/100 90/50	98 M 200 M	76 M 70 M	5.5 .02	§ 1	§ .01	§ .04	
66735*	42	11	5 AP 11 PP 165 PP	148/82 120/80 130/80	100 S 125 M 80 M	91 M 112 M 81 S	.1 Trace .27	1.8 47 0	.02 22.1   .19	.04 3.5 .48	
67966	31	0	24 AP 5 AP 9 PP 42 PP	162/102 210/140 145/100 146/100	160 M 77 M 93 M 104 M	148 M 89 M 81 M 63 M	.52 2.3 .16 .1	1.1 24 12 4	.21 .37 .12 .016	.06 .24 .22 .02	
71235	33	5	25 AP 9 PP 255 PP	150/100 120/90 138/86	74 M 107 M 88 M	82 M 110 M 59 M	.08 .03 .01	44 49 0	.51 7.0 .29	.34 21.7¶ .66	

TABLE I.—Continued

Case number	Age	Para	Days †	Blood pressure	Urea clearance ‡		Proteinuria	Addis count			Remarks
					1st hour	2nd hour		Casts	Red blood cells	White blood cells	
					per cent of normal	per cent of normal					
	years			mm. Hg			grams per 12 hours				
POSSIBLE RENAL INJURY											
52767*	22	3	24 AP 7 PP 245 PP 555 PP 3 AP	156/100 140/80 156/84 145/74	190 M 104 M 161 M 130 M	160 M 125 M 149 M 99 M	.26 .16 1.1 .36	125 73 52 97	2.0 .42 .75 .87	1.2 1.8 .56 .70	Pregnancy terminated at term. Sterilized
59342*	38	11	42 PP 165 PP 5 AP  50 PP 700 PP	260/150 250/90 220/140  190/120 230/140	92 M 135 M 80 S  60 M	88 M 100 S 80 S  50 M	  .8   50 M	  123  17.8	  .86  .08	  3.97  .20	Pregnancy terminated at 6 months
66142	40	6	14 AP 6 AP 11 PP 65 PP 440 PP	150/108 145/70 142/92 120/60 112/80	73 S 52 S 58 M 68 M 54 M	44 M 55 S 45 M 61 M 61 M	.53 .48 .42 .08 .01	§ 4 2 23 29	§ .04 .03 .24 .80	§ .04 .09 .18 1.06	
73692*	43	6	5 AP 17 PP 170 PP	140/90 110/90 110/76	91 S 103 S 98 M	80 S 155 S 94 M	.61 .17 .54	265 182 35	1.8 1.2 .32	2.0 .9 .4	
48129*	27	5	1 AP 248 PP	144/90 120/80	132 S 106 M	171 S 106 M	.8 .48	18 25	.92 .05	.82 .045	
76588	24	0	9 PP 450 PP	130/80 160/110	241 M 135 M	143 M 115 M	.05 Trace	15.3 24.9	.8 .45	3.1 .9	
84462	24	0	11 PP 150 PP	160/105 132/86	65 M 151 M	61 M 96 M	.36 .18	12.3 19.5	§ .87	§ 1.3	
40039*	24	3	10 PP 120 PP	135/90 112/70	75 S 69 M	72 M 56 M	Trace Trace	0 0	.92 .10	2.7 .41	
91845	29	0	210 PP	115/70	77 M	60 M	.36	0	.41	1.6	

\* History of previous preeclampsia.

† PP, Postpartum.

AP, Antepartum.

‡ M, Maximum clearance.

S, Standard clearance.

§ Alkaline urine.

|| Injury to urethra.

¶ Injected urine.

low them through repeated pregnancies in order to learn the effect of repeated toxemias on renal function, should the pregnancy or toxemia occur. The results also show that renal function tests are of no great value in the acute phase of the toxemia or early puerperium so far as diagnosis, prognosis or classification are concerned. They are an aid in prognosis in the period three months postpartum.

#### Eclampsia (Table II)

There were six cases of eclampsia, two of which are in the subgroup with no renal damage and

four are in the subgroup with possible renal injury. In only one case was there a history of previous preeclampsia. None gave a history of nephritis.

In the acute phase of the toxemia the blood urea clearance was normal in all cases. Too few observations were made in the early puerperium to be of value. However, in the group with renal damage one case had an abnormal clearance and in another the blood urea clearance was normal. In the period three months postpartum all of the cases with no renal damage had a normal blood

TABLE II  
*Renal function tests in eclampsia*

Case number	Age	Para	Days †	Blood pressure	Urea clearance ‡		Protein-uria	Addis count		
					1st hour	2nd hour		Casts	Red blood cells	White blood cells
	years			mm. Hg	per cent of normal	per cent of normal	grams per 12 hours	thousands per 12 hours	millions per 12 hours	millions per 12 hours
NO RENAL DAMAGE										
65299	28	3	13 AP 90 PP	140/96 126/80	94 M 132 S	90 M 166 M	.18 .05	13 10	.03 .11	.05 .11
70284	17	0	0 4 PP 10 PP 125 PP 360 PP	140/100 150/105 140/90 100/60 104/58	190 M 64 S 103 M 121 M	153 M 107 M 77 M 160 M 69 M	9.96 .89 .27 0 .18	2014 46 21 0 0	10,541 .58 2.75 .40 1.8¶	7.4 .72 1.6 .1 .27
RENAL DAMAGE										
43543	43	5	1000 PP	140/90	115 M	109 M	.2	10	1.08	.9
76931	22	0	8 AP 13 PP 70 PP	210/110 130/85 140/102	99 S 47 M 66 M	112 S 55 M 61 M	3.24 .36 .18	89 26 15	8.5 .28 1.65	755§ 1.03 16.5§
68108*	24	1	1 AP 11 PP 160 PP	150/100 110/90 100/75	125 M 131 M 59 M	118 M 125 M 72 M	3.3 .41 1.4	3636 802 37	2.3 1.9 .3	7.1 25.0 .75
45013	27	1	690 PP 900 PP	166/110 110/60	64 M	72 M 77 M	Tr. .36	20 7.2	.16 .06	.24 .40

\* History of preeclampsia.

† AP, Antepartum.

PP, Postpartum.

‡ M, Maximum clearance.

S, Standard clearance.

§ Urine infected.

¶ Trauma to urethra.

urea clearance while in the group with possible renal injury all observations save one were below the normal values established by Van Slyke.

The degree of proteinuria is of little significance during the acute phase of the toxemia or in the early puerperium so far as indicating the degree of renal involvement is concerned, since in all cases except two the values obtained in the period three months postpartum were within normal limits.

The Addis count during the acute phase of the toxemia was definitely abnormal in all cases. The number of all formed elements was extremely high in Cases 70284 and 68108, and all varieties of casts were noted. During the early puerperium there was a decrease in the number of

casts but the number was still abnormal. In the period three months postpartum all of the cases with evidence of renal damage had cast counts above the normal number established by Addis. The erythrocyte, leukocyte and epithelial cell counts were all high in the acute phase. In the period postpartum the red blood cell count remained above the limits of normal in only one case.

As in preeclampsia the tests are of no great value during the acute phase of eclampsia so far as the extent of renal damage is concerned. They are of distinct value in the period three months postpartum, indicating whether or not the kidney has sustained damage.

*Nephritis complicating pregnancy (Table III)*

Seven cases of nephritis complicating pregnancy have been observed. Since nephritis complicating pregnancy manifests itself early in gestation the tests are of value in establishing the degree of renal injury. The limits of normal established by Van Slyke and Addis are used for comparison when interpreting the results obtained at this stage of pregnancy.

In the five cases which were terminated early in pregnancy there was subsequently no marked increase in the extent of renal damage. In two of the five cases there was some evidence of improvement. In one of the two cases which was allowed to go to term there was a definite decrease in the values obtained for the blood urea clearance. The other showed no marked change in urea clearance, that she sustained damage, how-

TABLE III  
*Renal function tests in pregnancy with nephritis*

Case number	Age	Para	Days †	Blood pressure	Urea clearance		Proteinuria	Addis count			Remarks
					1st hour	2nd hour		Casts	Red blood cells	White blood cells	
	years			mm. Hg	per cent of normal	per cent of normal	grams per 12 hours	thousands per 12 hours	millions per 12 hours	millions per 12 hours	
80081*	22	0	56 AP 265 PP	130/75 120/70	65 M 49 M	71 M 44 M	.72 .36	0 0	3.6	14.7	Bacteruria, many Bacteruria [W.B.C.]
84139**	30	1	10 PP 90 PP 220 AP 330 PO§	140/98 156/100 160/100	84 M 74 M 71 M	75 M 71 M 78 M	4.15 2.10 .07	58 51 66	1.6 .92 .36	.55 .69 1.2	Pregnancy terminated at 2 months
76312*	20	0	53 AP 12 PP 102 PP	125/70 128/80 130/80	68 M 42 M 114 M	60 M 33 M 84 M	.25 .08 .06	84 47 41	.52 5.3 .5	.9 2.1 .5	
73792**	45	3	4 AP 18 PO§ 180 PO§	140/90 110/80 110/76	91 M 155 M 98 M	80 M 103 M 94 M	.61 .17 .54	265 181 35	1.8 1.2 .32	2.6 2.5 1.1	Pregnancy terminated at 6 months
5132**	28	4	3 AP 220 PO§ 410 PO§	150/110 150/108 170/110	45 S 37 S 42 M	46 S 35 S 40 M	.26 1.08	12 118	.05 .54	.1 .26	Pregnancy terminated at 4 months
83107*	38	4	30 AP 265 PO§	165/90 170/100	59 M 60 M	72 M 62 M	Trace .03	0 27	.265 .9	.26 .4	Pregnancy terminated at 2½ months
71141*	35	2	2 AP 19 PO§ 240 PO§ 420 PO§ 740 PO§	190/120 134/88 190/110 180/110 190/110	32 S 43 S 46 M 64 M 83 M	28 S 44 S 43 M 61 M 99 M	5.67 .30 .36	540 20 17	5.56 .3 .3	2.8 .7 .23	Pregnancy terminated at 5 months

\* History of nephritis.

\*\* History of nephritis and preeclampsia.

† AP, Antepartum.

PP, Postpartum.

‡ M, Maximum clearance.

S, Standard clearance.

§ Postoperative.

All of these women gave a history of nephritis and in addition three gave a history of a previous preeclampsia. Pregnancy was terminated early in five of the seven cases. The other two cases were not terminated because in one instance the nephritis was not severe enough and a child was wanted; the other was not terminated because of the patient's refusal.

ever, is indicated by the increase in the number of casts.

## DISCUSSION

The values obtained for the blood urea clearance tests and the Addis urinary sediment count in pregnant women with preeclampsia during the acute phase, prior to delivery and in the early

puerperium are of little significance so far as diagnosis and treatment are concerned. Three or more months postpartum the patients have apparently returned to the normal state so far as urinary findings and renal function are concerned. Recently Hundley et al. (5) have shown that the dilatation of the ureters and renal pelvis which usually occurs during pregnancy, returns to normal about six weeks postpartum. Three months postpartum the tests are of definite aid in prognosis and as an indication for further treatment. To obtain a better picture of this group of patients it would be necessary to follow them through repeated pregnancies over a period of years should the pregnancy with preeclampsia recur. By this procedure a proper evaluation of the cumulative effects of preeclampsia on renal function could be obtained, providing no intervening infection affecting the kidneys occurred.

In the cases of eclampsia, as in those of preeclampsia, the tests are of value for purposes of diagnosis and treatment only after the three month period postpartum. In those cases which sustain damage the extent seems to be of greater magnitude than in preeclampsia.

In the cases of nephritis complicating pregnancy the renal lesion was not found to have progressed following the early termination of the pregnancy. This is in contrast with the serious damage to the kidneys usually observed when such patients are allowed to go to term. In some of the cases which were terminated early there seems to be some improvement in the renal status. The tests have the same significance in pregnancy complicated by nephritis as in nephritis alone. The normal values established by Van Slyke and Addis were used for comparison in nephritis complicating pregnancy during its early stages.

## CONCLUSIONS

Measurements of the blood urea clearance and counts of the number of formed elements in the urinary sediment were made in thirty-three pregnant women with the various toxemias of pregnancy. The studies were done during the acute phase, in the early puerperium and three or more months postpartum or following operation for the termination of pregnancy. So far we have found the tests to be of no great value during the acute phase and in the early puerperium in cases of preeclampsia and eclampsia, though they have some diagnostic and prognostic significance in the period three months postpartum. In this period it was found that about fifty per cent of the cases of preeclampsia and sixty-seven per cent of the cases of eclampsia had sustained evidence of renal damage. The tests are of value in pregnancy complicated by nephritis, the values observed having the same significance as in nephritis alone. There is no increase in the extent of renal damage following the early termination of pregnancy.

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# PARATHYROID HORMONE IN THE BLOOD OF PREGNANT WOMEN<sup>1</sup>

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Histological changes indicative of hyperactivity have been found in the parathyroid glands of pregnant women by several investigators (1, 2). It has further been shown (3, 4) that it is possible to extract from large amounts of blood of pregnant women a substance that behaves pharmacologically like parathyroid hormone; this substance was not found in the blood of non-pregnant women.

Some years ago we described a method, since somewhat modified, for the demonstration of small amounts of parathyroid hormone (5, 6), and it was found that the hyperparathyroidism of rickets could be demonstrated by the use of only 30 cc., or less, of the blood from rachitic rabbits (7). The method is, briefly, as follows. If calcium chloride is administered at regular intervals to rabbits by stomach tube, one finds, generally, that the rise in serum calcium becomes less and less after each administration. If, however, parathyroid hormone, or any substance containing it in sufficient amounts, is given to the animals intramuscularly before the experiments, the rise in blood calcium is quite marked also after the later administrations of calcium chloride by mouth. The figure by which the results are judged is the difference between the rabbit's blood calcium before the experiment and the highest value obtained three or five hours later. This method has been successfully used not only by us but also by Shelling and Remsen (8) and by Dyer (9). The latter author points out that the rise obtained is not proportional, in the individual case, to the amounts of parathyroid hormone injected. We agree with Dyer on this point; just as in the dog, the amount of reaction varies in different rabbits, and we do not think that accurate quantitative information can be obtained from single experiments. Quantitative differences can, we believe,

be studied only by using sufficiently large groups.

Our results are summarized in Table I. The first column gives the rise of the rabbit's serum

TABLE I

*Rise in the serum calcium of rabbits after the injection of blood from 30 normal, non-pregnant, 74 pregnant and 11 lactating women*

Rise	Normal	Pregnant				Lactating
		Less than 15 weeks	15 to 24 weeks	25 to 34 weeks	More than 34 weeks	6 to 8 weeks
<i>mM. per liter</i>						
0.00 to 0.09 *....	27	8	6	1	7	10
0.10 to 0.19.....	1	7	2	3	3	
0.20 to 0.29.....	2	1	3	2		
0.30 to 0.39.....			6	1	2	
0.40 to 0.49.....			2	1	1	1
0.50 to 0.59.....			4	6		
0.60 to 0.69.....			5	3		

\* Cases with a decrease instead of a rise are included in this group.

calcium after the injection of woman's blood; the following columns give the number of cases showing the rise of calcium indicated by the first column. It may be seen that of the 30 non-pregnant cases 27 gave either a decrease, no rise or a very small rise in the rabbit's serum calcium. Quite similar results were obtained with the blood from women pregnant less than 15 weeks; the figures fall within the limits obtained in non-pregnant women. In the next two groups, however, (15 to 24 weeks and 25 to 34 weeks) we find a considerable number of cases where abnormally great rises were obtained, indicating that during this period of pregnancy an increase in the amount of parathyroid hormone present in the blood is rather common. Towards the end of pregnancy, however (sixth column), these cases are much less common, only 3 out of 13 cases showing an abnormal rise, and still more rare does such a re-

<sup>1</sup> Read before the Fiftieth Session of the Association of American Physicians at Atlantic City, May 1935.



sponse seem to be during the period of lactation included in our study.

We have also calculated the mean rise for each of the four periods of pregnancy listed in Table I.

were the direct cause of the effect observed on the blood calcium of the rabbit, some of these hormones were injected into rabbits, following the same technique as in the tests for parathyroid

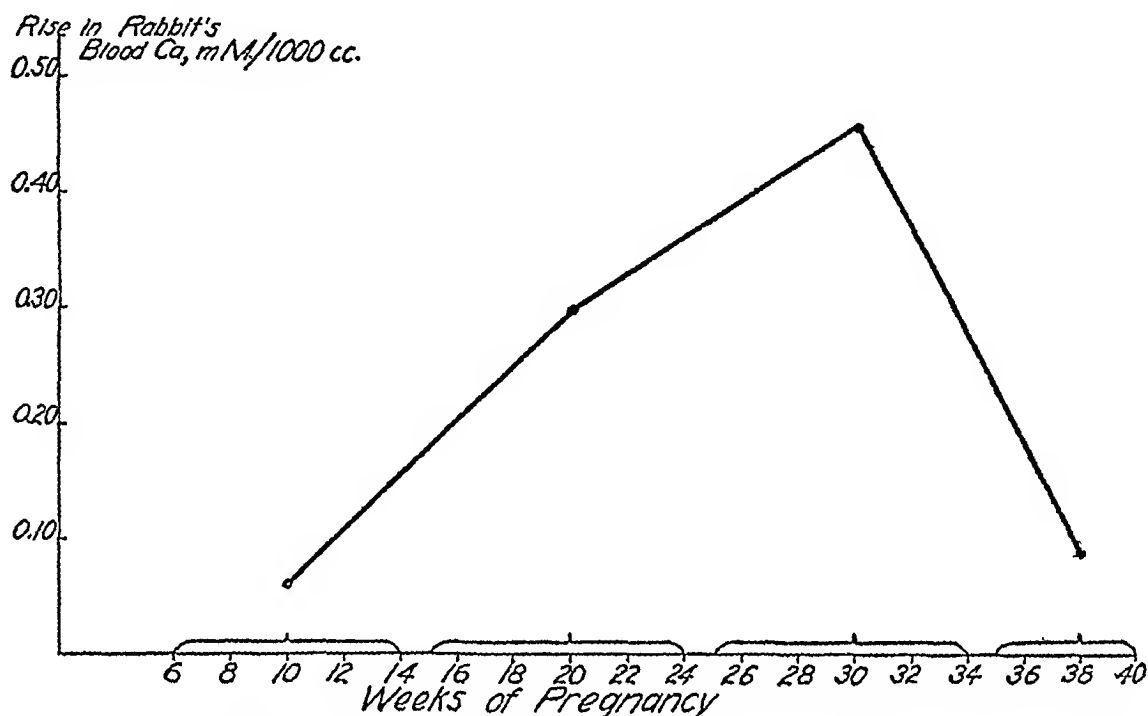


FIG. 1. AVERAGE RISE IN THE SERUM CALCIUM OF RABBITS AFTER THE INJECTION OF BLOOD FROM PREGNANT WOMEN (74 CASES).

A curve based on these means is shown in Figure 1, and as we have convinced ourselves that the difference between each two succeeding points on this curve is statistically significant, we think that we are justified in concluding that this curve gives an approximate idea of the degree of hyperparathyroidism during different periods of pregnancy. It must be pointed out, however, that according to Hoffmann (3, 4) the most potent extract can be prepared from the blood of women in the tenth month of pregnancy. Our findings indicate (Table I, Figure 1) that the blood is richer in parathyroid hormone during the seventh month than at any other time, and that in the tenth month the hormone content has usually returned to normal amounts. In order to study this matter further, we have made some observations on a pregnant dog (Figure 2); in this dog, just as in the women, we found the hormone content of the blood returning to normal just before delivery.

In order to exclude the possibility that other hormones which may be present in the blood of pregnant women in more than normal amounts

hormone. The results are tabulated in Table II, and it may be seen that none of the substances tested effected a rise in blood calcium at all comparable with that obtained by the injection of

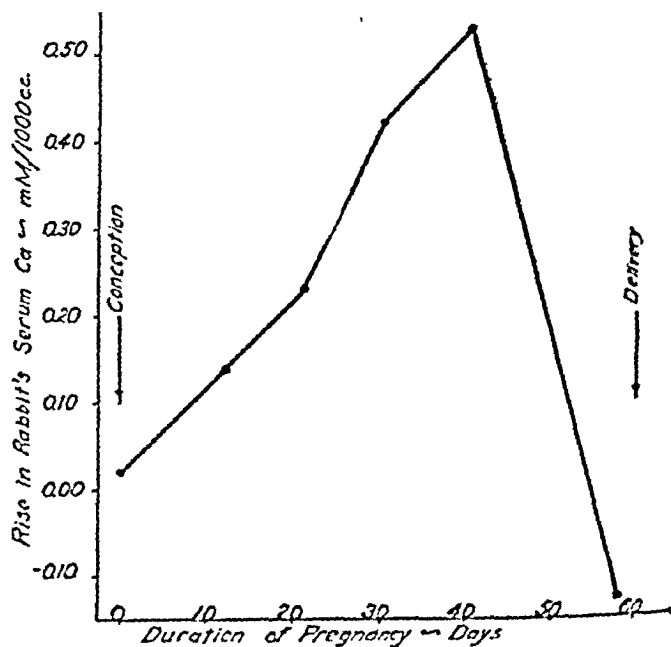


FIG. 2. RISE IN THE SERUM CALCIUM OF RABBITS AFTER THE INJECTION OF BLOOD FROM A PREGNANT DOG.

TABLE II

Rise in the serum calcium of rabbits after injection of various hormones

Hormone	Amount injected	Rise in rabbit's blood calcium* mM. per liter
Theelin (Parke and Davis)	0.5 cc.	0.03
Water solution	1.0 cc.	0.04
	1.5 cc.	0.00
	2.0 cc.	0.06
Theelin, in oil	1.0 cc.	0.08
Prolan	Extract from 83 cc. of urine	0.20
	Extract from 83 cc. of urine	0.05
	Extract from 83 cc. of urine	0.04
Thyroxin	2 mgm.	0.00 0.10
Adrenalin	0.04 mgm. per kgm.	-0.02
	0.03 mgm. per kgm.	0.27
	0.04 mgm. per kgm.	-0.08

\* When blood from women, pregnant 25 to 34 weeks was injected, the average rise was 0.46 mM. per liter (Figure 1); individual cases caused a rise to 0.60 to 0.69 mM. per liter (Table I).

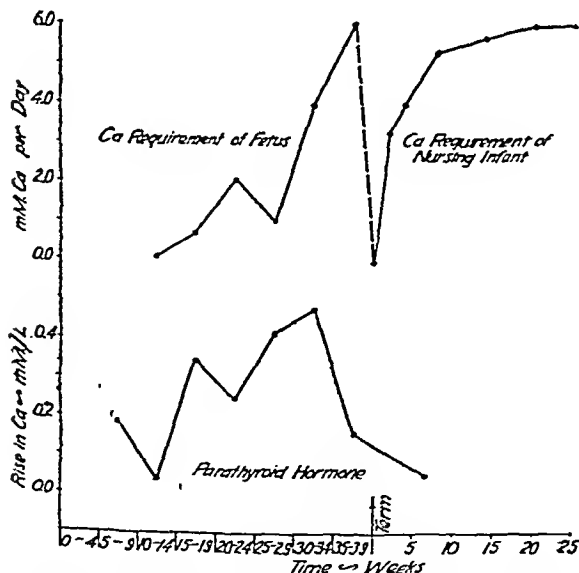


FIG. 3. COMPARISON BETWEEN CALCIUM REQUIREMENTS OF FETUS AND THE PARATHYROID HORMONE CONTENT IN THE BLOOD OF PREGNANT WOMEN.

The calcium requirements of the fetus are calculated from the figures given by Iob and Swanson (20), those of the nursing infant from average figures given in current handbooks. The average hormone content in the blood has here been calculated for periods half as short as those used in Table I and Figure 1.

blood from pregnant women. However, we do not know whether any of these substances might, indirectly, by stimulating the parathyroid glands, have been responsible for the increased amounts of parathyroid hormone found in the blood.

#### DISCUSSION

In Figure 3 a comparison has been made between the calcium requirements of the fetus and the nursing infant, and the parathyroid hormone content of the blood of pregnant and lactating women. At first these curves show a rough parallelism, on the whole they are both rising until the 34th week of pregnancy. At that time, however, they diverge sharply, the hormone content returning to normal while the calcium requirements show a marked increase.

It is possible that the parallelism of these two curves during the greater part of pregnancy indicates a causal relationship; that the increasing demands of the growing fetus with respect to calcium furnish a stimulus to the parathyroid glands. If that is true, we would have to assume that during the last weeks of pregnancy and during lactation, this stimulus, for some unknown reason, ceases to be effective, and it is interesting to note that during this period there seems to be some tendency towards true tetany with lowering of the serum calcium (10 to 19). It is customary to ascribe such a tendency to less than normal activity of the parathyroid glands. Our studies do not give any information as to whether the parathyroid hormone content in the blood is normal or less than normal. It is probable, however, that in the presence of an abnormal calcium metabolism (as in rickets) or unusual demands on the calcium metabolism (as in pregnancy) an insufficient amount of hyperactivity might also result in tetany.

In conditions such as pregnancy or rickets the hyperactivity of the parathyroid glands is accompanied by a serum calcium that, generally, is normal but occasionally shows a tendency towards low values. We believe that the hyperparathyroidism in these cases is of a different significance than in osteitis fibrosa cystica, where the serum calcium is high. Perhaps it would be a useful concept if we divided the various conditions in which hyperparathyroidism is found into two

## MATERIAL AND METHODS

The entire amount of urine for a period of 33 days was collected from a healthy male student<sup>2</sup> 18 years of age. As soon as 2 to 3 liters had accumulated the specimen was subjected to the procedure previously described (1), with the following exceptions. In each instance the primary ether extract after washing with water was repeatedly extracted with small amounts of 10 per cent HCl. The 10 per cent HCl solutions were united and kept in the refrigerator during the period of further collection and primary extraction of the urine. All of the hydrochloric acid extract was then covered with a large amount of ether, an excess of sodium acetate was added, and the mixture at once vigorously shaken. Having made certain that the aqueous fraction was negative to Congo paper, ether extraction was repeated until the porphyrins were removed completely. The further treatment of this solution of ether was the same as described previously (1). The final coproporphyrin solution in ether was not dried over sodium sulphate as was done previously, since it has been found to be true that a moderate loss of porphyrin occurs by adsorption on this salt. This was first noted by H. Fischer and Zerweck (11). The ether was simply decanted into a dry flask and was then removed completely upon the water bath. The porphyrin residue after drying in the air was esterified with HCl in methyl alcohol, and the ester was crystallized from chloroform-methyl alcohol in the usual way (1).

Employing the same method, porphyrin esters were isolated from the urines of the following cases.

*Case 1.* Fever. Male, 37 years of age with lung abscess and empyema. The daily elevation of temperature ranged from 101 to 103° F. Urobilinuria was 8.3 mgm. urobilinogen per day (23). There was no jaundice. Moderate anemia was present, and hemoglobin was 55 per cent (Sahli method, 17 grams per 100 cc. equivalent to 100 per cent). Urine was collected for a period of 12 days.

*Case 2.* Acquired hemolytic jaundice. Female, 18 years of age with slight icterus, without anemia, of 3 years' duration, increasing icterus and anemia, 4 months.

<sup>2</sup> The writer is indebted to Mr. Samuel Schwartz for the collection and preliminary extraction of this urine.

Hemoglobin was 32 per cent (Sahli), erythrocytes 1,150,000 per cu. mm. Stained smears revealed marked macroanisocytosis, polychromatophilia. Reticulocytes varied between 15 and 32 per cent. Resistance of erythrocytes to hypotonic saline was as follows: H<sub>1</sub> .38, H<sub>2</sub> .32, control H<sub>1</sub> .38, H<sub>2</sub> .30.

There was marked autoagglutination of erythrocytes in vitro (below 30° C.), the icterus index was 30, and the Van den Bergh reaction was of the indirect type. Feces urobilinogen: 1106 mgm. per day. (Normal range 100 to 250.) Urine urobilinogen 2.5 mgm. per day. (Normal range 0.5 to 3.0.) (23)

Physical examination revealed nothing except a smooth cystic mass in the left side of the pelvis. The spleen and liver were not palpable. It was considered possible that chronic hemorrhage into an ovarian cyst with local formation of bilirubin might be responsible for the findings described above. Consequently the cyst was removed at operation. It contained 800 cc. of a dark brown fluid rich in hematin (identified by hemochromogen spectrum after treatment with ammonium sulphide). Bilirubin and porphyrins were not identified, although searched for.

There was no improvement following this operation. On the contrary, icterus and anemia increased, and there was moderate fever. The urobilinogen in the urine increased markedly for a few days after operation, then decreased rapidly again. The highest value noted was 380 mgm. per day. During this period the urine was examined for porphyrins and a considerable increase of coproporphyrin was observed.

After an interval of three weeks during which the patient's condition remained precariously stationary, splenectomy was carried out. The spleen weighed but 460 grams, exhibiting microscopically a marked pulp congestion. The liver appeared to be entirely normal. This operation was followed by prompt improvement. Two months after operation the hemoglobin was 70 per cent (Sahli), the icterus index 10, and the feces urobilinogen 206 mgm. per day. Eight months later the patient reported that she felt entirely well and that there was no jaundice.

The urine employed for the isolation of coproporphyrin consisted of the entire amount for a period of four days immediately following the first operation (coincident with the highest urobilinogen excretion). This urine was first employed for the isolation of crystalline urobilinogen, a procedure which will be described elsewhere (24). Suffice it to say that this entailed a preliminary extraction with petroleum ether in which coproporphyrin is insoluble. Following this, the coproporphyrin was extracted with ether in the manner already referred to (1). It is worthy of note that this urine contained another pigment resembling bilirubin, but differing from it in that it failed to give a Gmelin test as well as the more delicate reaction for bilirubin devised by Harrison, and described by Godfried (12). Also differing from bilirubin the substance could not be extracted with chloroform from the urine. However, it gave a delayed and an indirect Van den Bergh reaction, and was precipitated by a barium chlo-

ride solution. In dilute NaOH ill defined absorption between 536 to 477  $m\mu$  was observed. Attempts to crystallize the substance were unsuccessful, probably because of its obvious lability, and it can only be stated that its characteristics as noted above appeared to be those of the "hämorubin" of Jenke (13), which, it is noteworthy, was likewise obtained from the urine of an individual with hemolytic jaundice.

*Case 3.* Lead poisoning. Male, 67 years of age. Mild anemia, hemoglobin 69 per cent. Considerable basophilic stippling of erythrocytes. Urine collection for a period of 5 days.

*Case 4.* Lead poisoning. Male, 46 years of age. Colic, anemia, hemoglobin 66 per cent, marked basophilic stippling of erythrocytes. Urine collection for a period of 4 days.

*Case 5.* Lead poisoning. Male, 38 years of age. Weakness of extensor muscles, mild anemia, hemoglobin 67 per cent, considerable basophilic stippling of the erythrocytes. Urine collection over a period of 6 days.

Cases 3, 4 and 5 were members of a large group of foundry employees suffering from lead poisoning.<sup>2</sup> The bearings in this foundry contained lead and were believed responsible for the numerous instances of lead poisoning which were encountered.

The total amount of urine collected in each of the above instances was subjected to the method of isolation already referred to. It is important to note that the fractionation of porphyrins embodied in this method depends upon characteristic solubilities (1, 2). Consequently, the coproporphyrins isolated and described in the following paragraphs possessed in each instance the typical solubilities of a coproporphyrin, particularly in not leaving 0.2 per cent HCl for chloroform. The sodium salt in each instance was entirely soluble in 10 per cent NaOH, a further characteristic of coproporphyrins.

Spectroscopic studies were carried out by means of apparatus and methods described previously (1, 2). Slight variations in the maxima for the absorption bands of the coproporphyrins isolated will be noted, if compared with those observed before (1, 2, 3). Since the spectrometer was not

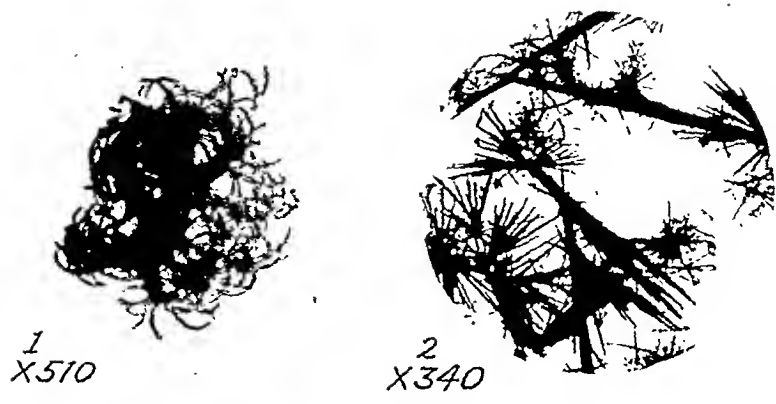
recalibrated, these are believed attributable to very slight changes in the relation of the wave length scale of the instrument to its grating. Of chief importance is the identification by means of superimposition of spectra, employing a pure coproporphyrin to compare with that under investigation. With the apparatus used (1), this permits detection of the slightest differences in position of absorption bands.

#### RESULTS (SEE TABLE I)

##### *Normal urine*

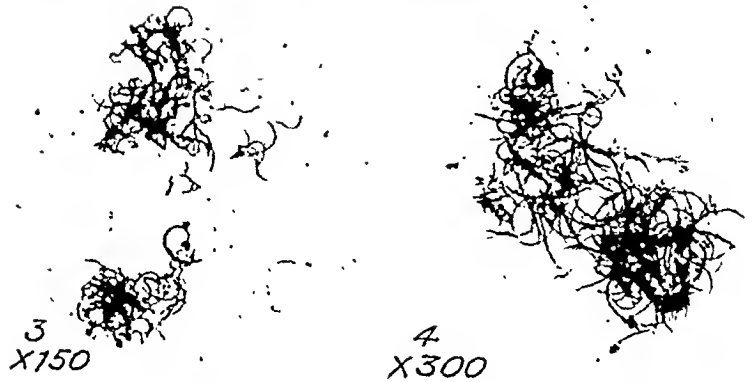
After four recrystallizations 0.13 mgm. of porphyrin ester were obtained from 33 days' normal urine. This will be spoken of as Fraction a. The ester composing this fraction crystallized rapidly in the form of fluffy masses consisting of fine curving and branching needles. This manner of crystallization is very characteristic of coproporphyrin I ester while coproporphyrin III ester crystallizes much more slowly in smaller clumps of crystals often adhering to the walls of the tube. These have the form of needle-like prisms which are usually broader than those of coproporphyrin I, and do not show the curving and branching of the latter. The crystals of coproporphyrin III ester rather frequently arrange themselves in concentrically united groups, or rosettes (10, 14, 15, 16). During crystallization and first recrystallization of Fraction a. it was observed that the mother liquor obviously contained more porphyrin than is usually true when coproporphyrin I ester is crystallized from chloroform methyl alcohol. These two mother liquors were combined and allowed to concentrate slowly at room temperature. After standing several days a further separation of crystals appeared. These were in the form of small aggregates part of which adhered to the walls of the small test tube in which the solution was contained. This material will be spoken of as Fraction b; its amount was evidently considerably less than that of Fraction a, nevertheless it was possible to recrystallize it twice in order to further observe its manner of crystallization; the amount did not suffice for a melting point determination. The characteristics of these fractions were as follows.

<sup>2</sup> The writer is indebted to Dr. E. C. Emerson of St. Paul, Minnesota, for the opportunity to investigate the urine of these cases.



1  
X510

2  
X340



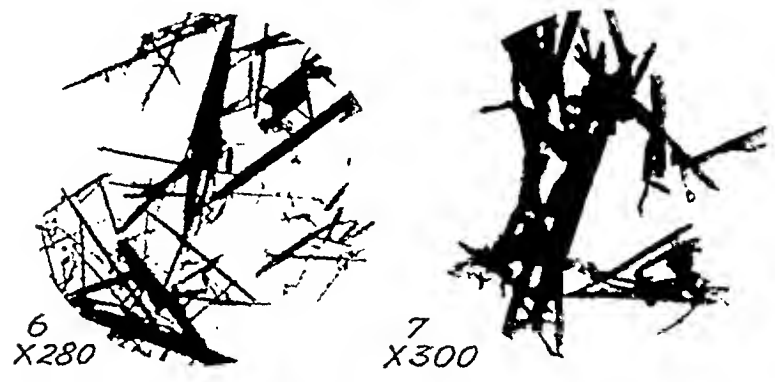
3  
X150

4  
X300



5a  
X150

5b  
X150



6  
X280

7  
X300

FIG. 1. COPROPORPHYRIN I ESTER FROM NORMAL URINE.

FIG. 2. COPROPORPHYRIN ESTER FROM NORMAL URINE, CRYSTAL FORM SUGGESTIVE OF COPROPORPHYRIN III.

FIG. 3. COPROPORPHYRIN I ESTER FROM URINE OF PATIENT WITH FEVER DUE TO LUNG ABSCESS AND EMPYEMA.

FIG. 4. COPROPORPHYRIN I ESTER FROM URINE OF PATIENT WITH ACQUIRED HEMOLYTIC JAUNDICE.

FIG. 5. COPROPORPHYRIN III ESTER FROM URINE OF FIRST CASE OF LEAD POISONING.

FIG. 6. COPROPORPHYRIN III ESTER FROM URINE OF SECOND CASE OF LEAD POISONING.

FIG. 7. COPROPORPHYRIN III ESTER FROM URINE OF THIRD CASE OF LEAD POISONING.

Fraction a. The crystals had the form of long curving needles (Figure 1). On recrystallization these appeared rather quickly in the light fluffy clumps characteristic of coproporphyrin I ester. After four recrystallizations the amount (0.13 mgm.) sufficed for one melting point determination. The substance did not melt sharply; shrinking was observed above 190°, melting from 218 to 223°. (Coproporphyrin I methyl ester, for which the writer is indebted to Professor H. Fischer in Munich, melted at 245 to 246° C.) No change whatever was exhibited at the lower temperatures critical for coproporphyrin III ester (142°, 172°). The absorption spectrum in acetic and ether solution was: I.  $\frac{627.8 - 622.2}{624.8}$ ; II.

faint maximum 597.5; III.  $\frac{582.7 - 568.3}{577.4}$ ; IV.  $\frac{533.5 - 526.0}{530.0}$ ; V.  $\frac{507.0 - 487.0}{496.6}$ . Order of intensity: V, IV, I, III, II. This was identical with the superimposed absorption spectrum of known coproporphyrin I in similar solution.

Fraction b. The crystals of this fraction appeared very slowly in the manner already referred to. The majority of the crystals were long narrow prisms similar to those of coproporphyrin III ester; a few curved and branching needles of the coproporphyrin I type were also noted. After two recrystallizations the appearance of crystals was still delayed and not prompt as is usual with even very small amounts of coproporphyrin I ester. The crystals now appeared to be uniform in type, namely, needle-like prisms frequently in groups (Figure 2), strongly suggestive of coproporphyrin III methyl ester. Spectroscopically this material was a coproporphyrin, having the following absorption spectrum: I.  $\frac{627.1 - 622.3}{624.9}$ ; II. faint maximum 597.9; III.  $\frac{581.8 - 568.0}{577.2}$ ; IV.  $\frac{534.0 - 525.2}{531.0}$ ; V.  $\frac{505.7 - 487.7}{496.9}$ . Order of intensity: V, IV, I, III, II. This was identical with the superimposed spectrum of a similar solution of coproporphyrin I.

### Pathological urines

Case 1. Fever due to lung abscess and empyema. After three recrystallizations 1.5 mgm. of porphyrin ester were obtained. This crystallized rapidly in the manner of coproporphyrin I, and the individual crystals were fine curving needles (Figure 3). The melting point was 204 to 208° C. The remaining substance was recrystallized twice more, following which the melting point was 215 to 218° C. All that remained was now mixed with coproporphyrin I methyl ester of melting point 245 to 246° C., in an approximate ratio of one part of the former to four of the latter. The melting point of this mixture was 240 to 243° C. From this it is evident that while the porphyrin isolated was still slightly impure, it melted far above the melting point of coproporphyrin III ester, and did not depress the melting point of known coproporphyrin I ester sufficiently to suggest that it was another porphyrin. The absorption spectrum was that of a coproporphyrin, i.e., I.  $\frac{626.7 - 622.2}{624.8}$ ; II.  $\frac{583.3 - 567.6}{577.8}$ ;

III.  $\frac{534.9 - 525.5}{530.9}$ ; IV.  $\frac{506.4 - 490.3}{497.5}$ . Order of intensity: IV, III, I, II. This absorption was identical with that of coproporphyrin I when the spectra were superimposed in the comparison spectrometer.

During the isolation of the above coproporphyrin it was noted that another porphyrin remained in the ether and was not extracted by 1 per cent HCl, but was removed quantitatively by 10 per cent HCl. This porphyrin was chloroform soluble, and was readily removed from HCl solutions by chloroform. The amount was too small to be obtained in a crystalline form; it was possible, however, to examine the substance spectroscopically. The acetic and ether solution was bluish pink and exhibited the following absorption: I.  $\frac{634.7 - 625.6}{631.5}$ ; II.  $\frac{598.0 - 571.9}{580.9}$ ; III.  $\frac{554.8 - 530.5}{536.6}$ ; IV.  $\frac{514.8 - 492.0}{502.3}$ . Order of intensity: IV, III, I, II. This absorption spectrum is quite characteristic of protoporphyrin (15), and in fact complete identity with protoporphyrin was observed when the spectra were superim-

were observed simultaneously with the heightened excretion of coproporphyrin. A porphyrin was isolated from the urine in three cases of lead poisoning. The crystal form and melting point of the ester indicated clearly that this was, in each instance, coproporphyrin III.

Since this communication was submitted for publication, an important finding of Hoerbürger's, mentioned in his inaugural dissertation (21), has come to the writer's attention. Hoerbürger isolated a crystalline coproporphyrin from normal urine; the crystal form and pH fluorescence curve (22) were characteristic of coproporphyrin I. The amount obtained was presumably too small for determination of the ester melting point.

An extensive study of the urinary porphyrins has recently been reported by Dobriner (25). Coproporphyrin I was obtained in a variety of diseases, including hemolytic jaundice and pulmonary infection; this finding is confirmed in the present report. Dobriner found coproporphyrin I in the urine in a number of instances of liver disease, which is in keeping with the writer's previous report concerning cincophen cirrhosis. In this connection it is of considerable interest that Dobriner found coproporphyrin III in one instance of atrophic cirrhosis, and in one of pigment cirrhosis.

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# THE NORMAL DURATION OF THE ELECTROCARDIOGRAPHIC VENTRICULAR COMPLEX

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The duration of ventricular systole has aroused the interest of a number of workers since Garrod pointed out its relation to the heart rate in 1871. The duration of systole has been measured by various vascular, intracardiac, and apical pressure records, heart sound records and combinations of these, the work of Wiggers and Katz (1) and Lombard and Cope (2) furnishing an introduction to this literature. Venous pressure, arterial pressure, posture, exertion, nervous influences and drugs have been found to modify it experimentally. Cheer and Li (3), Bartos and Burstein (4), Bazett and Sands (5) and others have investigated the relation of the duration of the electrocardiographic ventricular complex to the duration of systole and have found them to be closely related but not identical. The most accurate mechanical means for measuring the duration of systole are not applicable to clinical cases or are difficult to use. The duration of the electrocardiographic ventricular complex, on the other hand, can be determined easily on suitable records, and it seems likely that this record of cardiac activity is as closely associated with the state of the myocardium itself, though perhaps not with the general circulation, as the measurement of systole by mechanical means.

The ventricular complex begins with the beginning of the  $Q$ -wave and ends at the end of the  $T$ -wave. Because "electrocardiographic ventricular complex duration" is an inconvenient term and because introduction of the word systole makes for ambiguity, it will be referred to in this paper as " $Q$ - $T$  time."

Cheer and Li (3) have published a formula for the determination of the normal  $Q$ - $T$  time in the recumbent position based on measurements from 75 normal men and 43 normal women. Fridericia (6) published a formula for the determination of  $Q$ - $T$  time on the basis of measurements on 50 individuals—men, women and children. White and Mudd (7) have published a scattergraph of 50

normal individuals ( $Q$ - $T$  time against the  $R$ - $R$  interval) without stating the accurate measurements. Their scatter agrees quite well with 190 cases which they collected from the literature. Bazett (8) has published similar figures from 2 male infants, 2 boys, 12 men, and 19 women. Several records under various conditions were taken on some of his patients. The formulas which he gives apparently include all the measures on these cases, although this is not definitely stated. Miki (9), on the basis of a small group of normals, confirmed Fridericia's formula. Fenn (10) with measurements on 10 patients published a formula which approximated Bazett's. Lian, Golblin and Baraige (11) used a formula without giving the data upon which it was based, which gave a much wider "normal" range of  $Q$ - $T$  time at slow normal pulse rates than at rapid normal rates.

In an attempt to use the formulas of Fridericia and Cheer and Li in the study of  $Q$ - $T$  time, it was found that the measurements on our cases in practically all instances exceeded the prediction from these formulas. Because of this it seemed advisable to study the duration in normal individuals again. It was decided to test some characteristics of individuals other than rate, for influence on the  $Q$ - $T$  time.

## METHOD

A series of normal individuals was collected comprising 50 males and 54 females. Standard lead electrocardiograms were obtained on a Hindle string galvanometer electrocardiograph with a time marker controlled by a tuning fork. This time marker, by repeated checks against a stop watch, was found to have an error of approximately  $1\frac{3}{4}$  per cent. All figures as given have been corrected for this error. Four more tracings were taken but discarded either because the base line was so irregular that accurate measurement was impossible (3 cases), or because



ventricular extrasystoles were found (1 case). The entire series was presumably normal; it consisted of medical staff, nurses, students and secretarial workers. A physical examination was performed on all cases. It may or may not be significant that they all considered themselves to be in good health, and were not "patients without cardiac disease." All tracings were taken with the subjects supine, but no effort was made to control the extent of previous activity. No effort at selection according to rate or any other characteristic was made, aside from checking for normality. The film speed was increased to about 5 cm. per second with the thought that the increased speed might facilitate accurate measurement. The impression gained was that it did not. In both Leads I and II at least 15 consecutive cardiac cycles, exclusive of the standardization, were recorded. A shorter strip of Lead III was taken. Leads I and II were measured separately. The pulse interval recorded was the average of 15 consecutive *R-R* intervals. The duration of systole was measured by projecting the film in a photographic enlarger on to graph paper ruled in squares of  $\frac{1}{10}$  of an inch. It was enlarged so that .01 second on the record corresponded to  $\frac{1}{10}$  of an inch. It is the author's impression that this makes it possible to measure the record with accuracy as great as the inevitable slight irregularities of the base line will permit. The points were marked on the graph paper and measured later. This prevented any tendency to modify measurements because of a knowledge of previous measurements. No effort was made to be more accurate than .005 second in the location of any point. It is probable that an attempt at greater accuracy is not justified in the great majority of records. No effort has been made to evaluate exactly the error in measurement, but the author believes that it is less than .01 second in suitable records. In the series of 50 males, 15 consecutive complexes were measured in each of the first two leads because of the statement by Lombard and Cope (2) that this was necessary in order to get a true average measurement. The average measurement of the first 5 of each set of 15 was then compared with the average of the 15. The maximum difference between these averages was .006 second, this difference occurring twice in

50 cases. The average difference was .0018 second. The means, standard deviations and regression formulas were not significantly different. On the basis of this comparison only 5 complexes were measured in each lead in the series of females and the average of 5 used in both series.

Several other data were collected on each subject including height, weight, and the age at the nearest birthday. In the electrocardiogram the axis was determined, the height of the *T*-wave was measured, and measurements of three parts of the ventricular complex were made as follows: first, the time from the beginning of the *QRS* complex to the peak of the *R*-wave; second, the time from the peak of the *R*-wave to the peak of the *T*-wave; and third, the time from the peak to the end of the *T*-wave.

The data obtained were treated statistically, the male series slightly more extensively than the female series because some of the factors considered were obviously without influence on the *Q-T* time in the former series and were therefore not considered in the latter. The method of the statistical work will be outlined only briefly. In the male series Pearsonian linear zero order correlation coefficients were determined between all possible combinations of seven variables. The variables used were the *Q-T* time, the *R-R* interval, the electrocardiographic axis, the *T*-wave height, the height, the weight, and the age. Multiple correlations between *Q-T* time and various combinations of the other variables were computed by Horst's (12) modification of the Doolittle method. Three measures of *Q-T* time were used—that obtained from Lead I, that obtained from Lead II, and one obtained by selecting whichever *Q-T* time average measure was the longer from Lead I or II. In each case the average *R-R* interval and *T*-wave height from the corresponding lead was used.

The female series was treated the same way except that height of the *T*-wave and electrocardiographic axis were not included. The same procedures were carried out with the combined groups except that only Lead II and whichever lead had the longer *Q-T* time were used, Lead I not being considered separately.

Some calculations were made using the time

from the peak of the *R*-wave to the peak of the *T*-wave rather than *Q*-*T* time.

Scatter diagrams were constructed to ascertain the type of functional relationships between the characteristics. For the purposes of this study it was found that these relationships could be satisfactorily represented by straight lines, and consequently linear correlation analysis was used.

Table I shows the individual measures of *Q*-*T*

TABLE I

*Q*-*T* time (the longer measure of Lead I or II—Average of 5 complexes) and pulse interval (the average of 15 complexes from the corresponding lead)

Males					Females						
Number	Q-T*	R-R†	Number	Q-T*	R-R†	Number	Q-T*	R-R†	Number	Q-T*	R-R†
1	.376	.837	27	.377	1.049	1	.365	.713	32	.391	.729
2	.344	.617	28	.380	.933	2	.392	.853	33	.386	.747
3	.367	.910	29	.383	.923	3	.361	.698	34	.378	.856
4	.402	.937	30	.344	.722	4	.391	.815	35	.377	.833
5	.369	.771	31	.379	.869	5	.392	.810	36	.372	.793
6	.347	.686	32	.375	.914	6	.432	.965	37	.386	.761
7	.368	.744	33	.331	.595	7	.380	.723	38	.402	.902
8	.377	.818	34	.359	.704	8	.373	.788	39	.388	.924
9	.379	.768	35	.332	.647	9	.377	.798	40	.405	.813
10	.388	.903	36	.364	.750	10	.381	.807	41	.370	.762
11	.367	.775	37	.376	.900	11	.376	.804	42	.380	.867
12	.369	.836	38	.387	.912	12	.385	.793	43	.384	.818
13	.345	.692	39	.356	.752	13	.397	.876	44	.368	.694
14	.364	.708	40	.427	.945	14	.413	.853	45	.368	.894
15	.428	1.081	41	.361	.764	15	.410	.897	46	.443	1.256
16	.351	.813	42	.364	.668	16	.383	.888	47	.376	.943
17	.407	.969	43	.358	.786	17	.392	.717	48	.382	.914
18	.364	.756	44	.361	.727	18	.348	.777	49	.386	.916
19	.365	.724	45	.355	.813	19	.386	.873	50	.362	.776
20	.354	.814	46	.393	.885	20	.367	.815	51	.373	.829
21	.399	.964	47	.371	.876	21	.386	.754	52	.353	.777
22	.428	1.113	48	.382	.914	22	.331	.679	53	.387	.769
23	.384	.842	49	.370	.866	23	.376	.735	54	.415	1.011
24	.384	.741	50	.363	.759	24	.365	.691	55	.422	1.118
25	.382	.491	51	.363	.759	25	.389	.847	56	.356	.823
26	.365	.668	51	.373	.856	26	.365	.765	58	.396	.807
						30	.388	.955	59	.363	.694
						31					

\* *Q*-*T* = *Q*-*T* time in seconds.

† *R*-*R* = *R*-*R* interval in seconds.

time and *R*-*R* interval; in each case these measures are taken from Lead I or II, the lead with the longer average *Q*-*T* time being used. These are the original measurements upon which the recommended formula is based. Some of the other data considered are summarized in Table II.

#### DISCUSSION

The regression formulas obtained in the correlation analysis express the relationship between characteristics, and therefore are a device for estimating one characteristic from one or more other characteristics.

Two selections are to be made—first, the measure of *Q*-*T* time to be predicted, and second, the

other individual characteristics to be used in making the prediction. As stated above three measures of *Q*-*T* time and two other measures of part

TABLE II

Means, standard deviations and correlation coefficients

Variable	Sex	Lead	Mean	Standard deviation	Zero order correlation coefficients			
					<i>R</i> - <i>R</i>	Age	Height	Weight
<i>Q</i> - <i>T</i> time, seconds	M	C*	.3723	.0213	+.827	+.005	+.212	+.061
	M	I	.3626	.0209	+.795	+.000	+.268	+.156
	M	II	.3705	.0225	+.792	+.023	+.211	+.068
	F	C	.3332	.0178	+.738	-.077	+.123	+.176
	F	I	.3745	.0199	+.827	-.038	+.235	+.157
	F	II	.3822	.0199	+.705	-.038	+.312	+.223
<i>R</i> - <i>T<sub>p</sub></i> , seconds	M	I	.2397	.0153	+.534	-.008	+.225	+.033
	M	II	.2449	.0178	+.847	+.028	+.162	+.019
	F	I	.2615	.0171	+.613	+.016	+.153	+.119
	F	II	.2633	.0175	+.600	-.008	+.207	+.146
<i>Q</i> - <i>T<sub>p</sub></i>	M	I	.2750	.0154	+.783	-.016	+.235	+.120
<i>R</i> - <i>R</i> pulse interval, seconds	M	C	.8207	.1145		-.170	+.065	+.043
	M	I	.8274	.1133		-.133	+.015	+.042
	M	II	.8141	.1140		-.133	+.049	+.019
	F	C	.8231	.1045		-.265	+.265	+.167
	F	I	.8311	.0992		-.217	+.278	+.173
	F	II	.8264	.1056		-.276	+.327	+.174
Age, years	M		23.75	4.90			+.059	+.165
	F		27.74	6.05			-.281	+.153
Height, cm.	M		178.9	6.2				+.613
	F		162.7	5.7				+.200
Weight, kgm.	M		73.10	9.71				
	F		56.23	6.20				

\* C = the lead (I or II) with the longer *Q*-*T* time.

† *R*-*T<sub>p</sub>* = the time from the peak of *R* to the peak of *T*.

‡ *Q*-*T<sub>p</sub>* = the time from the beginning of *QRS* to the peak of *T*.

of the electrocardiographic ventricular complex were considered. The *Q*-*T* time in Lead II is longer than in Lead I in the majority of cases and its average is larger (Table II). In some instances, however, the *Q*-*T* time is definitely longer in Lead I than in Lead II. If this divergence is marked and the rates in the two leads are approximately the same, it is found that the Lead II measure is markedly shorter than the prediction. The obvious explanation for this finding is that part of the ventricular complex is isoelectric in Lead II, either the beginning of the *QRS* or the end of the *T*. In this series most of the cases in which the duration in Lead I was longer had a *Q*-wave in Lead I but not in Lead II. This suggests that the first portion of the electrocardiographic ventricular complex is isoelectric in Lead II in these cases. Probably the discrepancies between the *Q*-*T* time measures are all due to isoelectric phases of the complex in one lead or the other if the rates are the same. This

is a source of error that can be partially ruled out by taking the longer of the two measures, regardless of the lead in which it occurs. When the beginning of *QRS* is isoelectric in one lead and the end of *T* isoelectric in another lead the total *Q-T* time would not be found by selecting the longer of the two. On the basis of the theory of Einthoven's triangle (precordial leads were not taken in this series) any electric effect in the heart must be registered in two of three leads, and therefore one of the three leads would necessarily show deviations from the isoelectric line at both ends of the ventricular complex. In occasional instances this might be Lead III. The *Q-T* time in Lead III was measured in all cases in which the longer of the measures from the first two leads showed a significant negative deviation from prediction. In no instance was the measure found to be longer in Lead III. It is probably advisable to choose the longest *Q-T* in any lead, however, in measuring the *Q-T* time, because presumably we want to measure the total duration of ventricular electrical activity. In both the male and female series the correlation between *Q-T* time and pulse interval is slightly higher when the longer measure between Leads I and II is used than when either Lead I or II measures are used alone (Table II). The mean of the *Q-T* measure is only slightly longer when the longer of the two *Q-T* measures is used (Table II).

As mentioned previously, the *Q-T* time was divided into three parts, that is, the time from the peak of *R* to the peak of *T* and the time preceding and the time following this interval. The primary reason for this was to determine whether the measurement from the peak of *R* to the peak of *T* could be used to greater advantage than the total *Q-T* time. It is easier to obtain an accurate measure because both points are relatively clean cut. If this or some other measure of the ventricular complex could be predicted with greater accuracy than *Q-T* time a new problem as to its usefulness would be presented. The correlation coefficients between the measurements between the peaks of the *R* and *T*-waves and the pulse interval are somewhat higher in males and lower in females than the corresponding correlation coefficients between *Q-T* time and pulse in-

terval (Table II). This indicates that no definite increase in accuracy can be obtained by using the distance between the peak of *R* and the peak of *T* instead of the *Q-T* time. The measurement from the beginning of *QRS* to the peak of *T* is also no better than *Q-T* time in its correlation with pulse interval (Table II). Meakins (13) studied the interval from the end of the *QRS* to the end of *T* but this measure is not considered here because of the difficulty in measuring it. From these studies it seems advisable to use a prediction formula using the longest *Q-T* time from any lead. In this series, Lead III offered no advantage so that the longer of Leads I and II was used. Three formulas were developed using this *Q-T* time measure and the pulse interval. For the male series the formula is

$$\overline{Q-T} = .1536 R-R + .2462$$

in which *Q-T* = *Q-T* time and *R-R* = pulse interval. The standard error of estimate of *Q-T* is .012 second. For the female series the formula is

$$\overline{Q-T} = .1259 R-R + .2789$$

and the standard error of estimate is .014 second. For the two groups together the formula is

$$\overline{Q-T} = .1464 R-R + .2572$$

and the standard error of estimate is .014 second.

To facilitate comparison of these formulas they have been plotted (Figure 1). It will be noted that the predicted or average *Q-T* time for corresponding pulse intervals is longer in females than in males. With a pulse interval of 1.10 seconds (rate 55) the predicted *Q-T* time for females is .002 second longer than for males, while with a pulse interval of .60 second (rate 100) it is .016 second longer. Statistical criticism shows that the difference between the male and female formulas is great enough to indicate a high probability of true sex difference. (See below.) This concludes the consideration of the choice of the measure of *Q-T* time to be predicted.

The prediction of *Q-T* time from pulse interval alone is discussed above. Other measurements on each individual are height, weight, age, height of the *T*-wave, and electrical axis. In the male

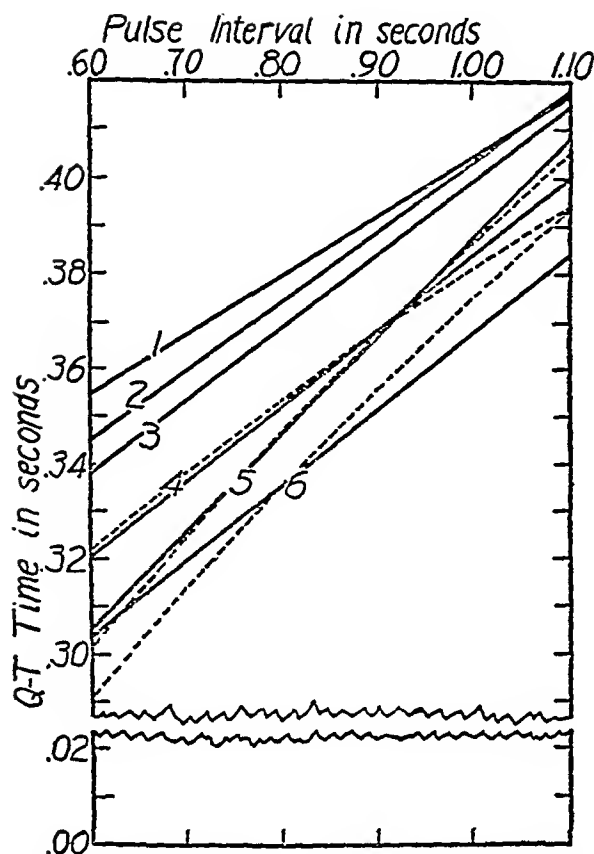


FIG. 1. REPRESENTATION OF FORMULAS FOR PREDICTION OF  $Q-T$  TIME FROM PULSE INTERVAL (REGRESSION LINES).

Lines 1 to 3 are from the author's series. Number 1—females:  $\bar{Q}-\bar{T} = .1259 R-R + .2789$ , in which  $\bar{Q}-\bar{T}$  =  $Q-T$  time in seconds and  $R-R$  = pulse interval in seconds. Number 2—sexes together:  $\bar{Q}-\bar{T} = .1464 R-R + .2572$ . Number 3—males:  $\bar{Q}-\bar{T} = .1536 R-R + .2462$ . The curved line formulas as published by other workers are represented by dotted lines 4 to 6 and the straight line formulas developed from the same data are represented by solid lines 4 to 6. Number 4—Fridericia (6)—sexes together—straight line:  $\bar{Q}-\bar{T} = .1599 R-R + .2245$ ; curved line:  $S = 8.22 \sqrt[3]{P}$ , in which  $S = Q-T$  time and  $P$  = pulse interval, both in hundredths of seconds. Number 5—Cheer and Li (3)—females—straight line:  $\bar{Q}-\bar{T} = .2079 R-R + .1799$ ; curved line:  $S = .3877 \sqrt{P}$ , in which  $S = Q-T$  time and  $P$  = pulse interval, both in seconds. Number 6—Cheer and Li—males—straight line:  $\bar{Q}-\bar{T} = .1605 R-R + .2069$ ; curved line:  $S = .374 \sqrt{P}$ .

series multiple correlations with these five observations as well as pulse interval were computed in an effort to increase the accuracy of the prediction of  $Q-T$  time. Height of the  $T$ -wave and electrical axis were dropped from consideration at once because their influence on the  $Q-T$  time

was obviously negligible. This judgment was made on the basis of a comparison of the partial  $\beta$  regression coefficient of each variable with its standard error. The values of these in Lead II are given in Table III. Some insignificant differences in the relationships are found when Lead I is used.

TABLE III  
Partial  $\beta$  regression coefficients and their standard errors (Lead II)

Independent variables used in estimating $Q-T$ time	Male				Female	
	7 variables		5 variables		5 variables	
	$\beta$	$\sigma_\beta$	$\beta$	$\sigma_\beta$	$\beta$	$\sigma_\beta$
Pulse interval.....	.792	.084	.809	.081	.720	.105
Age.....	.193	.086	.173	.082	.192	.111
Height.....	.296	.106	.295	.101	.125	.125
Weight.....	-.197	.111	-.214	.102	.004	.121
T-height.....	.072	.092				
Axis.....	.018	.093				

In the author's group of males, height, weight and age increased the accuracy of prediction to some extent, but the significance of these characteristics for this purpose in the general population was in doubt. Therefore multiple correlations with these variables were computed in the female series. The evidence from this group of females indicates that height, weight and age have little if any effect on  $Q-T$  time. When a formula is computed with the sexes together, the correlations of  $Q-T$  time with age, height and weight change from positive to negative which indicates that none of them has as great an influence as sex does, even though this is slight. This entire group is not very satisfactory from the standpoint of age, the range being from 20 to 48 years, with only 8 of the 104 patients over 35 years old. The evidence from this study does not demonstrate that weight,  $T$ -wave height, and electrical axis influence  $Q-T$  time. If age and height of the individual have any influence it is slight and of little use in the estimation of  $Q-T$  time. In addition it was found that height, weight and age do not increase the accuracy of prediction of other measures of the ventricular complex mentioned above more than they do the prediction of  $Q-T$  time. In no case is the standard error of estimate of  $Q-T$  time from a formula

drawn from multiple correlations more than .001 second smaller than the standard error of estimate from the corresponding formula using pulse interval alone.

The duration of the ventricular complex has never been predicted with complete accuracy from any set of variables because it does not vary in constant relation with any known combination of characteristics. Predictions will probably be subject to an error of about .01 second because of error in measurement in individual cases. The error in predicting  $Q-T$  time from the pulse interval is less than .01 second in more than half the cases. The errors in the remaining cases range up to .04 second with few errors in excess of .03 second. The usefulness of the  $Q-T$  time measure is impaired because of this wide deviation of a few cases, as White and Mudd (7) have shown. Further study is advisable to attempt to find other characteristics which will aid in the prediction of  $Q-T$  time.

This study is of interest chiefly because of the marked difference between the normal values for  $Q-T$  time as determined from this and previous series. Fridericia's (6) formula is:

$$S = 8.22 \sqrt[3]{P}$$

in which  $S = Q-T$  time and  $P =$  pulse interval, both in units of .01 second. Cheer and Li (3), Bazett (8) and Fenn (10) used formulas of the form:

$$S = K \sqrt{P}$$

in which  $S = Q-T$  time,  $K =$  a constant, and  $P =$  the pulse interval. Cheer (3) found  $K$  to be .374 in males and .3877 in females. Bazett (8) found it to be .37 in males and .40 in females. Fenn (10), on a mixed group, found  $K$  to be .39. These formulas are obviously curved lines when plotted. The curves, however, are slight, approximating straight lines through the normal range of pulse rates in adults. These writers have apparently used this type of curved line regression formula not because the relationship between  $Q-T$  time and pulse interval is a curved line, but because the increase of  $Q-T$  time with pulse interval is not proportional. As a number increases, its square root and cube root also increase but less rapidly. The increase of  $Q-T$

time with pulse interval bears a more nearly constant proportion to the increase of either the square root or cube root of pulse interval than to the increase of pulse interval itself. Fridericia gives mathematical justification for the use of the cube root type of formula rather than the square root type in his series of cases. Cheer and Li (3) state that the results of their studies confirm the square root type of formula without giving mathematical proof. The curved line regression formulas of Fridericia and Cheer and Li have been plotted (Figure 1). To make this type of formula accurate in all cases it would be necessary to use roots which are not integral numbers, which would complicate its use somewhat. The straight line type of formula as used above in the author's series (plotted in Figure 1) is adjustable to any data of this type.

The data used by Fridericia and by Cheer and Li in calculating their curved line formulas have been used in calculating formulas of the straight line type. These have been plotted (Figure 1). Fridericia did not divide his cases by sex, and it was not done in calculating straight line formulas because of the relatively small number of cases. As might be expected from his careful development the straight line formula approximates Fridericia's cube root formula, the two lines crossing twice (Figure 1). Between rates of 55 and 100 the greatest difference of prediction between the straight line and cube root formula is .003 second at a rate of 55. The standard errors of estimate of  $Q-T$  time of the two are practically identical. In the male series of Cheer and Li the slope of his curved line square root formula is definitely steeper than the slope of the straight line regression formula developed from the same data (Figure 1). The lines cross at a pulse interval of .80 (rate 75). At a pulse interval of 1.10 (rate 55) the prediction by the straight line formula is .010 second shorter than with the square root formula. At a pulse interval of .60 (rate 100) the prediction is .013 second longer by the straight line formula than by the square root formula. In the female series of Cheer and Li, which is smaller, the correspondence between the square root formula and the straight line formula is close with the lines crossing twice and a maximum difference between the two formulas in the range of normal

heart rates of .002 second at a rate of 100. It should be noted, however, in this connection that the average rate of the females is rather high and that the relatively few cases in the slower rate brackets affect the slope of the curve heavily. Perhaps this may account for the marked divergence of the straight line formulas at the slower rates, while they cross at a rate of slightly greater than 100. It will be noted that while the straight line formula of this series of females approximates the square root formula, the straight line formula of Cheer and Li's males is practically parallel with, although lower than, the straight line formula from Fridericia's cases.

The difference in prediction by the various formulas is striking. This expresses a difference between the measurements of  $Q-T$  time as compared with pulse interval in the three series of cases. There are three possible explanations for this difference: first, technical considerations—polarization in the circuit or inaccuracy in the time registration in some of the machines used; second, a difference between observers as to what constitutes the duration of the ventricular complex; and third, an actual difference between the different series of individuals. Polarization is not present in the author's records and, since it would probably tend to lengthen the  $Q-T$  time, is probably not an explanation for the fact that other measurements are shorter. In regard to the time registration nothing can be said except that the timer used in this series has been checked as stated above. The location of the end of the  $T$ -wave is sufficiently uncertain so that some individual difference in measurement might enter, but it seems almost impossible that this should be great enough to account for the discrepancy. As to the third possibility, that the difference is due to differences in the individuals used, it may be said that the chance that the differences between the three series represent errors incident to random sampling from the same population is so remote as to be discarded. The fact that each of the three series was drawn from different parts of the world from persons with different racial characteristics and modes of life may be important. No conclusions can be drawn regarding the reason for the discrepancy between the two prediction formulas on the basis of present data.

Cheer and Li found that females had longer average  $Q-T$  time measurements for corresponding heart rates than males. This was also the case in Fridericia's series, although the small series does not justify separation into sexes in calculating prediction formulas. Bazett also noted a sex difference as stated above. When the straight line formulas (Figure 1) of Cheer and Li are compared a different relationship is found to exist between the sexes than in the case in the author's series, although in both instances the prediction for females is longer than for males at corresponding rates. The male and female predictions approximate each other at a pulse interval of .60 second (rate 100) but are widely divergent (.027 second) at a pulse interval of 1.10 (rate 55). While the two lines of Cheer and Li converge in the range of rapid normal rates, the two lines of the author's sample converge in the slow normal rates. These findings support the conclusions made above that a true sex difference exists but no decision can be made as to whether this difference is greater for one heart rate than another. In other words, the difference between the heights of the sex lines is significant but the difference between the slopes is not (Figure 1).

In using either the curved or straight line type of formula a prediction of  $Q-T$  time for any given rate may be made and the deviation of the measured duration in a given case from the prediction for that rate obtained. It seems that this method gives a clearer, more easily handled conception than the calculation of " $K$ " for each case and the determination of the deviation of the individual " $K$ " from the normal " $K$ ." This determination of " $K$ " gives the deviation of measure from prediction a value in proportion to the size of the prediction, but the same result may be obtained by expressing the deviation of the measured duration from the predicted duration as a percentage of the prediction as was done by Berliner (14). However, there is no evidence at the present time to show that the percentage deviation is more significant than the absolute deviation.

The use of the formulas may be simplified by constructing a table. The value for each pulse interval is determined and entered opposite the pulse interval or the corresponding pulse rate. If it is desired to express deviations from prediction

as a percentage, the table may be amplified by another column showing the percentage of the prediction represented by .01 second and from this the percentage deviation of any measure from the prediction can be readily calculated. Table IV is such a table.

TABLE IV  
*Predictions of Q-T time for normal heart rates*

Pulse interval	Heart rate	Q-T time	
		Male	Female
<i>seconds</i>	<i>per minute</i>	<i>seconds</i>	<i>seconds</i>
.60	100	.338	.354
.65	92	.346	.361
.70	86	.354	.367
.75	80	.361	.373
.80	75	.369	.380
.85	71	.377	.386
.90	67	.384	.392
.95	63	.392	.399
1.00	60	.400	.405
1.05	57	.407	.411
1.10	55	.415	.417

SUMMARY

1. The most accurate formulas for the prediction of the duration of the electrocardiographic ventricular complex (Q-T time) from measurements of pulse interval in a series of 50 normal males and 54 normal females are: for males,

$$\overline{Q-T} = .1536 R-R + .2462$$

and for females,

$$\overline{Q-T} = .1259 R-R + .2789$$

in which Q-T = duration of electrocardiographic systole, and R-R = pulse interval. A table is provided which simplifies the use of these formulas.

2. The use of age, height, and weight of the individual, and height of the T-wave and axis of the electrocardiogram does not appreciably increase the accuracy of prediction of the duration of the electrocardiographic ventricular complex.

3. Other workers have found the duration of the electrocardiographic ventricular complex to be shorter for corresponding pulse rates. The reason for this difference is not apparent, but several possible explanations are discussed.

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# COMBINED CORD DEGENERATION WITHOUT ANEMIA: A CASE REPORT WITH STUDIES BEARING ON THE "INTRINSIC FACTOR" OF CASTLE

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The purpose of this paper is twofold: first, to report an unusual case of combined cord degeneration with achlorhydria but without anemia in which a prolonged spontaneous remission has been observed; and second, to describe some experimental observations bearing on the "intrinsic factor" of Castle. The case itself raises the question of the exact relationship of combined cord degeneration and pernicious anemia; the experimental studies raise the question of the exact rôle of the "intrinsic factor" of Castle in each of these conditions.

The prevailing view seems to be that in the absence of "ergotism, pellagra, syphilis, arteriosclerosis, and cachectic states" all patients with combined cord degeneration are in reality cases of pernicious anemia (1, 2, 3, 4, 5, 6). This is supported by the recognized frequency of combined cord degeneration in pernicious anemia, by the generally accepted opinion that the neurologic signs may precede the appearance of anemia by many months or years, and by the constancy of achlorhydria in both conditions.

It is universally agreed that in pernicious anemia degeneration of the spinal cord and brain frequently occur as a part of the disease process itself, but the incidence of such changes is estimated variously by different authors. Sturgis, Isaacs, Goldhamer, Bethell, and Farrar (7) in a recent review of the literature found estimates ranging from less than 25 per cent to 90 per cent or more, the majority of writers favoring a frequency of about 70 per cent.

In those cases in which an interval of time has occurred between the appearance of the neurologic manifestations and the anemia, the interval has been of variable duration. In Woltmann's series (4) the longest period was thirteen months; in Bramwell's case (8) it was three years; in Grinker's (9), four years, but in neither Bramwell's nor Grinker's reports are the descriptions of the blood findings quite adequate. There seem to be occasional cases of combined cord degeneration in which anemia does not develop over a period of years, if at all. Russell, Batten, and Collier (10) described the clinical course and autopsy findings in two such cases, one of four months' and the other of thirteen months' duration. In neither of these was anemia present. In the first case, the red blood cell count two days before death is given as 4,500,000 cells per cubic millimeter, hemoglobin, 90

per cent; in the second, no figures are recorded, but the statement is made, "There was no anemia." Ungley and Suzman (6), in their study of combined cord degeneration, noted six cases in which no definite anemia was present. The three patients who survived for more than two years without liver treatment had comparatively high counts, viz., 5.6, 4.45, and 3.9 millions per cu. mm. respectively. No further details are given with regard to these cases. Castle, Heath, and Strauss (11) refer to a similar case (Case C), but the symptoms were only of one month's duration. Allen (12) reported a case of combined cord degeneration without anemia, but it was only of ten months' duration. Vanderhoof (13) described six patients in whom the duration was longer, but the reports are incomplete. Wilkinson (14) states that he "has examined 39 cases of subacute combined degeneration without anemia," but further details are not given. Beebe and Wintrobe (15) describe a case of two years' duration, but the picture is somewhat obscured by the fact that liver therapy was given from time to time in spite of the fact that there was no anemia. Greenfield and O'Flynn (16) in reviewing forty-five cases of subacute combined cord degeneration, noted two cases in which the first red cell count was high, 5.5 million in one and 4.85 million in the other, but no further details are given. Sargent and Harris (17) mention having seen two cases of combined cord degeneration without anemia and report in detail one of two years' duration. In this case liver had not been given and yet the red blood count was 4.96 million, hemoglobin 88 per cent, color index 0.9. Sanford (27) cites three cases of cord degeneration of indefinite duration with a normal blood picture. All of these are said to have improved after the administration of liver therapy. Briggs and Oertling (28) refer to a case with a normal blood picture, but the published abstract does not contain the full details. It apparently has not been completely settled, therefore, whether in the absence of "ergotism, pellagra, syphilis, arteriosclerosis and cachectic states" combined cord degeneration with achlorhydria but without anemia must be considered as pernicious anemia or whether it may be a disease *sui generis*.

There is apparently a complete unanimity of opinion with regard to the invariable association of achlorhydria with combined system disease regardless of the presence or absence of anemia. Wilkinson (14) states that he has "fully confirm (ed) the findings of other observers that achylia gastrica exists in 100 per cent of such cases." Greenfield and O'Flynn (16) found achlorhydria to be a constant feature in their series of forty-



five cases. In this connection it is interesting to note that Sturgis, Isaacs, Goldhamer, Bethell, and Farrar (7), after weighing all the evidence, conclude that true pernicious anemia, irrespective of the cord changes, is invariably associated with true achylia gastrica. In a sense then, achylia gastrica may be considered as the constant link between the two conditions.

According to the concept of Castle (20) a macrocytic anemia should develop whenever there is an absence of "intrinsic factor" in the gastric secretion, unless there is an adequate intake of liver, liver extract, or other anti-anemic substances. On this basis Beebe and Wintrobe (15) suggested that the demonstration of the presence or absence in the gastric juice of the "intrinsic factor" of Castle might be employed as a diagnostic test for pernicious anemia. They were unable to demonstrate the presence of the "intrinsic factor" in two cases in which only a tentative diagnosis of pernicious anemia had been made and concluded therefrom that the "test" had substantiated the tentative diagnosis. Theoretically the principle involved here seems simple, but there are certain difficulties which have not yet been eliminated. Isaacs, Goldhamer and Sturgis (18) have demonstrated the presence of "intrinsic factor" in the gastric secretion in pernicious anemia by collecting and using quantities of juice comparable to that secreted by a normal stomach. They suggest that the deficiency of "intrinsic factor" is a relative rather than an absolute one. This observation is not incompatible with the work of Castle, but it raises the problem of the determination of the minimum amount of "intrinsic factor" compatible with a normal blood picture. Exact quantitative methods of measurement are not yet available, but it is quite probable that the maintenance amount will be found to vary in different individuals, particularly when such factors as diarrhea, malnutrition, and inadequate intake of food are present. Further difficulties arise from the work of Barnett (19), which is, in part, contradictory to that of Castle. Barnett found the "intrinsic factor" to be present in an apparent case of pernicious anemia, and on the other hand, failed to find it in simple achlorhydria without anemia. Hartfall and Witts (29) found the "intrinsic factor" present in variable amounts in simple achlorhydric anemia. In some cases the result was questionable, and Hartfall was forced to the verdict "that it is not proved that the gastric juice in simple achlorhydric anemia always contains the intrinsic factor of Castle in amounts comparable with normal."

Only four observations have been found in the literature with regard to the presence or absence of "intrinsic factor" in combined cord degeneration without anemia. Castle, Heath, and Strauss (11) reported a positive response with a reticulocytosis of 12.8 per cent on the tenth day. Beebe and Wintrobe (15) on the other hand failed to obtain a response. In the former case the symptoms were only of one month's duration; in the latter, the picture was complicated by intermittent liver therapy. Briggs and Oerting (23) reported obtaining a prompt but transitory reticulocyte response with no change in the

final blood picture. This was interpreted by them as indicative of "a deficiency of the anti-anemic (intrinsic) factor in subacute combined sclerosis despite the presence of a normal blood picture." The fourth observation is that of Reimann and Weil (32). These workers modified Castle's method for demonstrating the presence of intrinsic factor by substituting 10 to 20 grams of whole liver for the 200 grams of beef muscle. This enabled them to use smaller quantities of gastric juice and seemed to give results comparable to those obtained with Castle's technique. In the protocols introduced as evidence, Reimann and Weil cite two experiments in which they used the gastric juice of two patients with achylia gastrica and combined cord degeneration, but no further details are given with respect to these two cases. Reticulocyte responses to 11.4 per cent were obtained in the first instance and to 12.6 per cent in the second. There was a corresponding increase in the erythrocyte count and the hemoglobin.

It is apparent that the interrelationships of these various conditions: pernicious anemia, combined cord degeneration, achlorhydria, and the presence or absence of Castle's "intrinsic factor" are not entirely clear at the present time. Consequently, we have thought it worth while to describe a case of combined system disease without anemia and with marked spontaneous improvement under our observation for over two years, and to report in relation thereto our investigations with regard to the "intrinsic factor."

#### CASE HISTORY

The patient (Unit Number 41500), a sixty-one year old unmarried school teacher, was admitted to the Albert Merritt Billings Hospital August 23, 1933, complaining of progressive stiffness and weakness in the lower extremities of about fifteen months' duration and of complete inability to walk for five weeks. The diet had been general; attempts to elicit a history of dietary deficiency were unsuccessful. The general physical examination was negative. There was no lemon yellow tint or pallor of the skin and no atrophy of the papillae of the tongue. The past medical history consisted of scarlet fever, pertussis and measles in childhood, a dilatation of the anal sphincter in 1931, and a tonsillectomy in 1932. There was no history in any respect suggestive of venereal disease. The family history contained nothing noteworthy. The neurological examination by Drs. Percival Bailey and P. M. Levin revealed normal cranial nerves; fairly good motor strength and normal tone in the upper extremities; weakness of the lower extremities in all movements, more those of flexion than of extension, with no paralysis and with a moderate increase in the tone of the leg muscles. There was no muscular atrophy. Pain sensibility was acute everywhere with no level changes. Touch was slightly impaired over the lower extremities. Vibratory sense was lost over the lower extremities, pelvis and lower lumbar spine; intact in the upper extremities. Sense of position was defective in the toes and fingers, more in the former than the latter.

Impaired stereognosis was present in both hands. Two-point discrimination was slightly impaired in both hands. There was no ataxia or tremor in the finger-to-nose test with the eyes open or closed, but there was marked ataxia of the legs, worse with the eyes closed. The patient was unable to walk unaided and swayed markedly in the Romberg position. The biceps, triceps, radial, patellar and achilles reflexes were present and equal on the two sides; the upper and lower superficial abdominal reflexes were bilaterally absent; the Babinski and Hoffman signs were bilaterally present and positive. There was no deformity or tenderness of the spine. Impression: Posterolateral syndrome, probably due to subacute combined cord degeneration.

A spinal puncture was performed August 24, 1933. The spinal fluid was clear; the initial pressure 140 mm., promptly rising to 210 mm. upon compression of the right jugular vein; promptly falling upon release of the compression, but immediately rising again to 200 mm. upon compression of the left jugular vein; falling at once upon release, but promptly rising to 250 mm. upon compression of both jugular veins. The cell count was 5 lymphocytes per cc.; there was a faint trace of globulin; the Wassermann reaction was negative; the total nitrogen 0.24 gram per liter.

The blood Wassermann and Kahn tests were negative. The erythrocytes were reported as 4.76 million per cc., the hemoglobin was 90 per cent (Sahli), the leukocytes 8,700 per c.mm., and the smear described as normal. Repeated urinalyses were normal; there was no occult blood in the stool when the patient was given a meat-free diet. Roentgenologic examination of the stomach was reported as normal; the colon was large and described as a megacolon. The frequently repeated histamine tests of gastric secretion invariably revealed a complete achlorhydria. Gastroscopic examination of the stomach by Dr. Rudolf Schindler performed October 29, 1934, and repeated November 8, 1934, revealed a complete atrophy of the entire mucous membrane of the stomach.

*Course.* The patient remained in the hospital under constant observation for almost two years, and has remained in the neighborhood since her discharge from the hospital. During this time there has been very marked symptomatic improvement. With encouragement and training she has learned to walk almost unaided. Periodic neurologic examinations, however, have failed to disclose any significant change. There has certainly been no evidence of progression of the disease process. The blood count has been reported variously by the changing internes, but at no time have the red cells been less than 4.0 million per cc., or the hemoglobin less than 79 per cent (Sahli). Approximately two years after admission, on October 9, 1935, a hematologic investigation by Dr. Ernestine Kandel revealed a perfectly normal blood picture: erythrocytes, 4.46 million per c.mm.; hemoglobin, 84 per cent (Dare); leukocytes, 9,300 per c.mm.; the differential white count: polymorphonuclear cells, 54 per cent; small lymphocytes, 38 per cent; monocytes, 6 per cent; eosinophils, 1 per cent; basophils, 1 per cent;

smear, perfectly normal; cell volume 45; volume index, 1.08. The Price-Jones curve (May 15, 1935) was perfectly normal, the peak occurring at 7.4 micra, with a normal distribution.

The patient received no specific therapy—no hydrochloric acid, no liver, and no iron, with the exception of 12 cc. of Lederle's liver extract (each 3 cc. containing the active principle derived from 100 grams of liver) intramuscularly within the first ten days after admission and 4.0 grams of ferrous carbonate, given in a two-day period six weeks later in October, 1933. These did not appear to have any immediate effect and were given empirically before a policy of prolonged observation without specific treatment had been decided upon.

The diagnosis is apparently that of subacute combined cord disease with achlorhydria, but with a normal blood picture, although over three years have elapsed since the onset of the disease.

### *Experimental procedures*

Experiments were designed to test for the presence in the gastric secretion of the anti-anemic "intrinsic factor." Following the method of Castle and his coworkers (20), gastric juice, following histamine stimulation (.0007 gram), was obtained daily from this patient. Samples were collected every ten minutes for one and one-half hours and pooled. The amounts of secretion thus obtained daily varied from fifty to one hundred twenty-five cubic centimeters. Castle was able to obtain an average of only forty cc. of gastric juice daily while studying a similar case. At the end of each collection period, one hundred cc. of 0.5 per cent HCl was introduced into the stomach by means of the Rehfuess tube and removed in fifteen minutes. The collected secretion and the washing were pooled and then incubated with two hundred grams of finely ground lean beef at a pH between 2.5 and 3.5 for two hours. The material was run through a fine sieve and neutralized to a pH of 5.0.

This extract was given daily for ten days to a sixty-two year old female patient, Unit Number 24714, with the classical symptoms and findings of pernicious anemia without cord changes. The result is shown in Figure 1. On the day following the conclusion of the experiment, the experimental subject was given nine cc. of Lederle's liver extract intramuscularly. There was a typical reticulocyte rise to 18 per cent five days later, preceded by an increase in the red blood count which continued to rise after the reticulocytosis disappeared. We interpret this as a negative response in so far as Castle's extract is concerned.

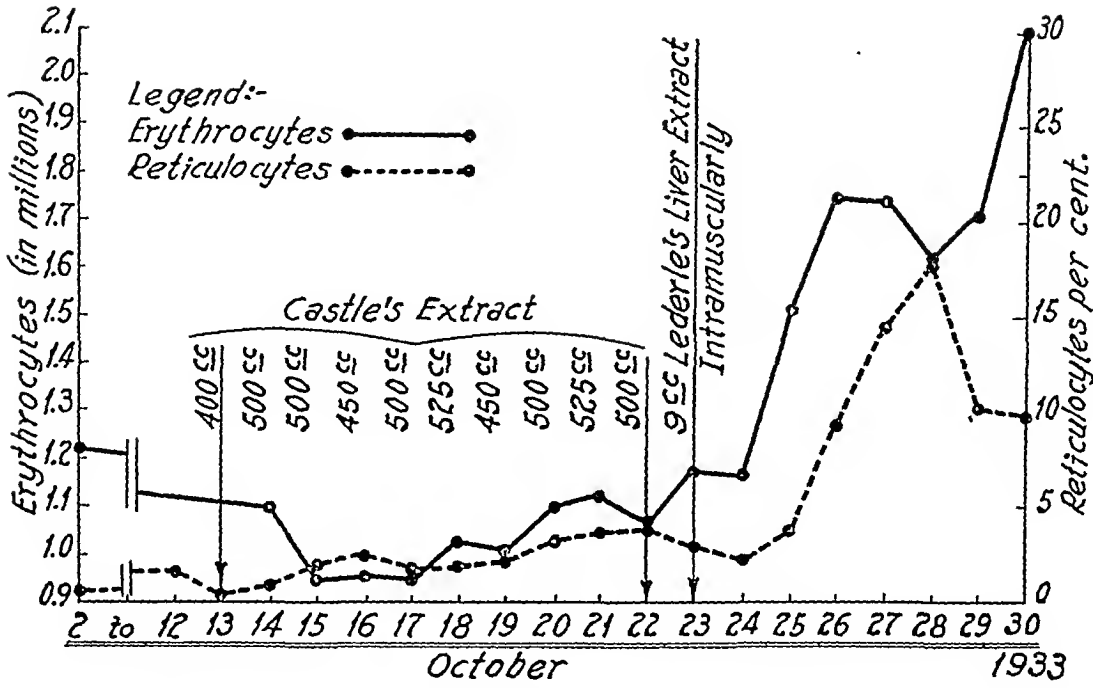


FIG. 1. NEGATIVE RESPONSE WITH EXTRACT OF PATIENT'S GASTRIC JUICE AND BEEF. UNIT NUMBER 24714.

because the reticulocyte count did not exceed 4 per cent until the third day after the intramuscular injection of liver extract, and the erythrocytes did not rise appreciably until the third day after the conclusion of the experiment, the second day after the injection of the liver extract. According to the usual standards, this should be considered a negative experiment, but the early rise

in the red blood count after the injection of liver extract and slightly prior to the reticulocytosis suggests that Castle's extract did contain very small amounts of hematopoietic material, and hence presumably very small amounts of intrinsic factor.

Two months later an opportunity arose to repeat the experiment. The test subject was a

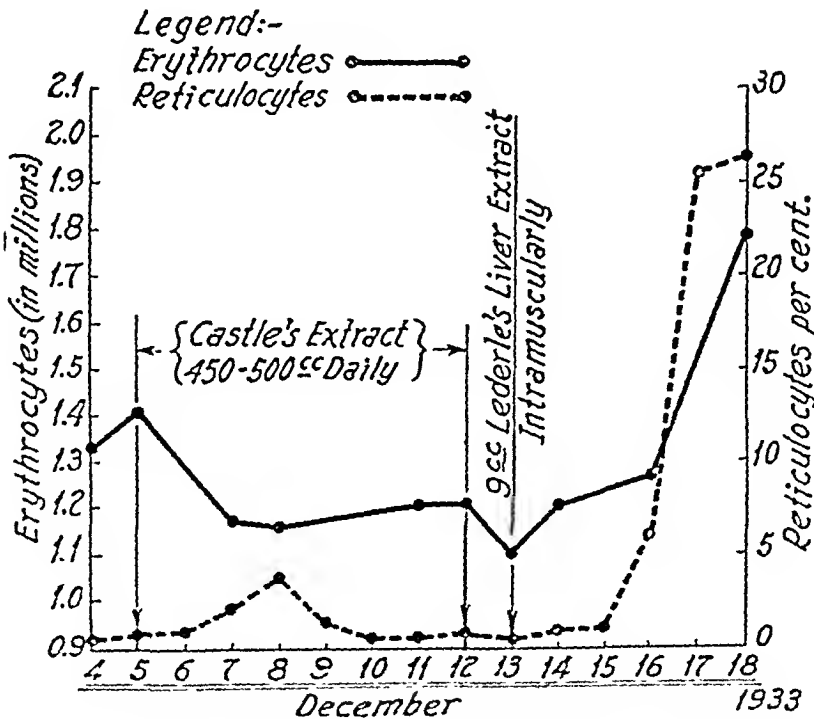


FIG. 2. NEGATIVE RESPONSE WITH EXTRACT OF DIGEST OF PATIENT'S GASTRIC JUICE AND BEEF. UNIT NUMBER 94242.

male patient, Unit Number 94942, forty-five years of age, with the classical symptoms and findings of pernicious anemia without cord changes. The extract was given for eight days. On the ninth day the patient became so concerned over his lack of improvement that it was necessary to give him parenteral liver extract (Lederle). A typical reticulocyte response appeared three days later, presumably attributable solely to the liver extract, as shown in Figure 2. This experiment is also interpreted as a negative response.

### Controls

Negative experiments of this kind in our opinion may be considered properly as of little value unless controlled in such a fashion as to prove the

to Castle's extract when normal human gastric juice and beef are incubated, extracted, and fed to a test subject with pernicious anemia. In this case the test subject was a woman (Unit Number 81754), age sixty-four, who had been under observation and treatment in another institution for five years, during which time the course had been typically that of pernicious anemia with relapses whenever the therapy lapsed as a result of her failure to maintain it. Death subsequently occurred from carcinoma of the stomach—a case falling, in our judgment, into the group described by T. Grier Miller (21) and by Conner and Birkeland (21) and others. The reticulocyte level was rather high, but the rise of both the reticulocytes and the erythrocytes following the

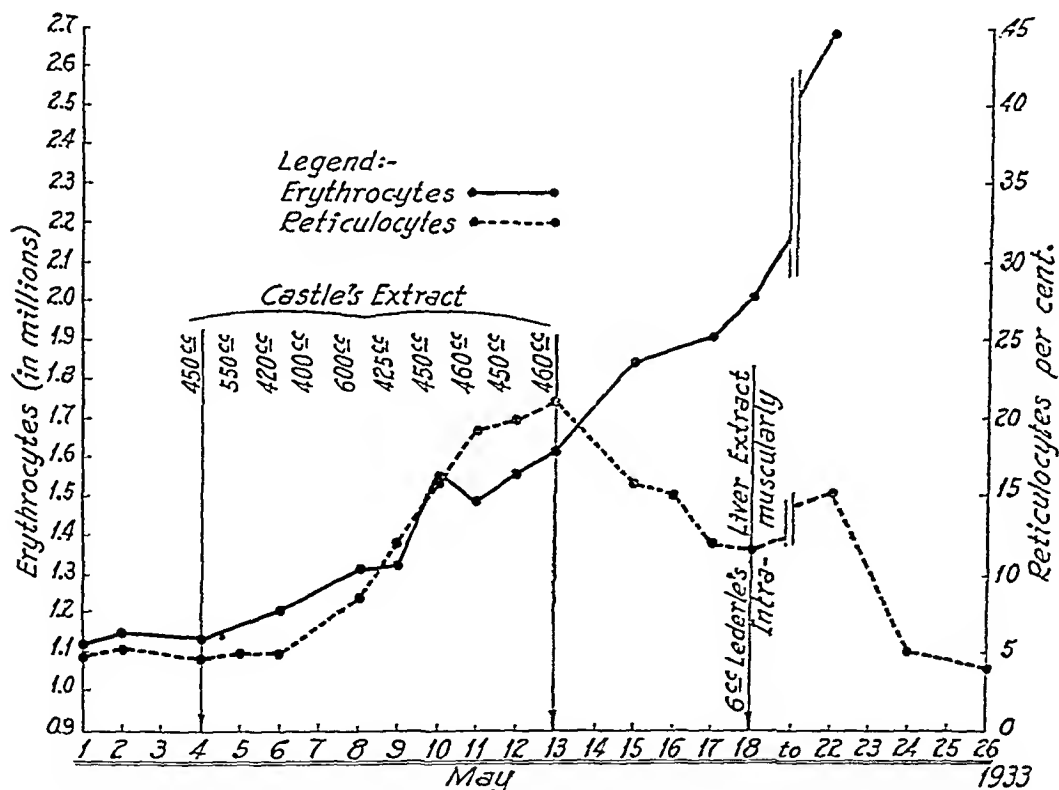


FIG. 3. CONTROL EXPERIMENTS: POSITIVE RESPONSE WITH EXTRACT OF DIGEST OF NORMAL HUMAN GASTRIC JUICE AND BEEF. UNIT NUMBER 81754.

correctness of the technique employed. For this reason we have felt it necessary to include charts of three control experiments in which exactly the same procedure was carried out. Figure 3 shows the classical reticulocyte and erythrocyte response

administration of Castle's extract was very marked and prompt.

Figure 4 shows a somewhat different type of response to a similar extract of normal human gastric juice and meat fed to another patient

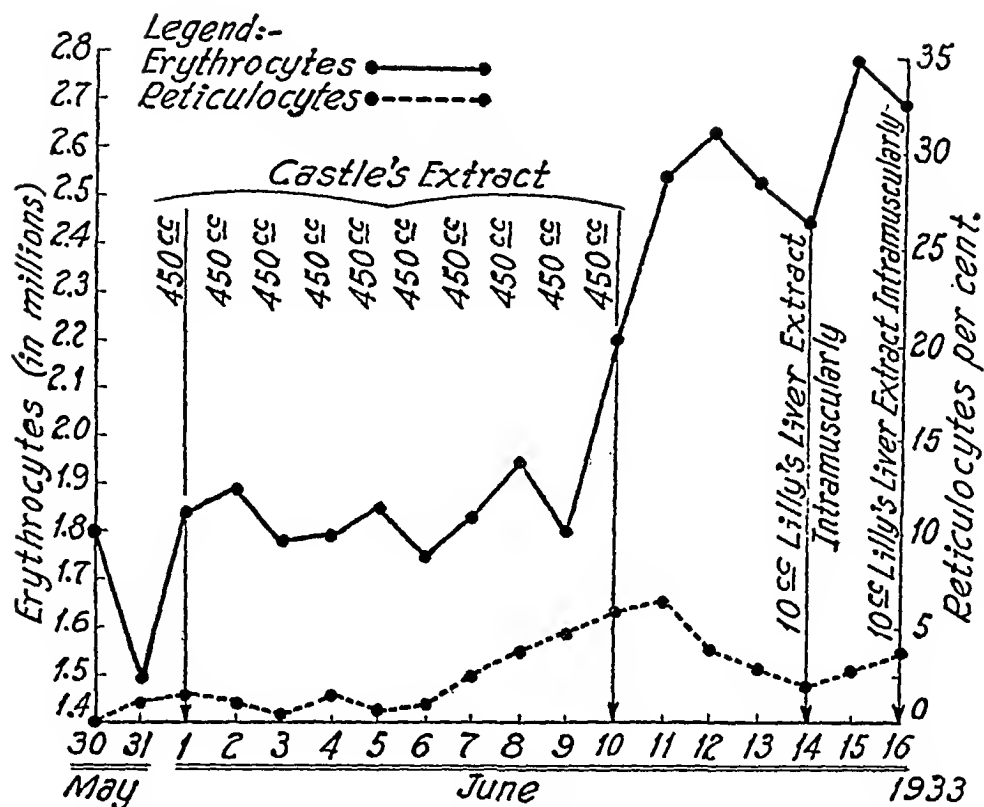


FIG. 4. CONTROL EXPERIMENT: POSITIVE RESPONSE WITH EXTRACT OF DIGEST OF NORMAL HUMAN GASTRIC JUICE AND BEEF. UNIT NUMBER 62087.

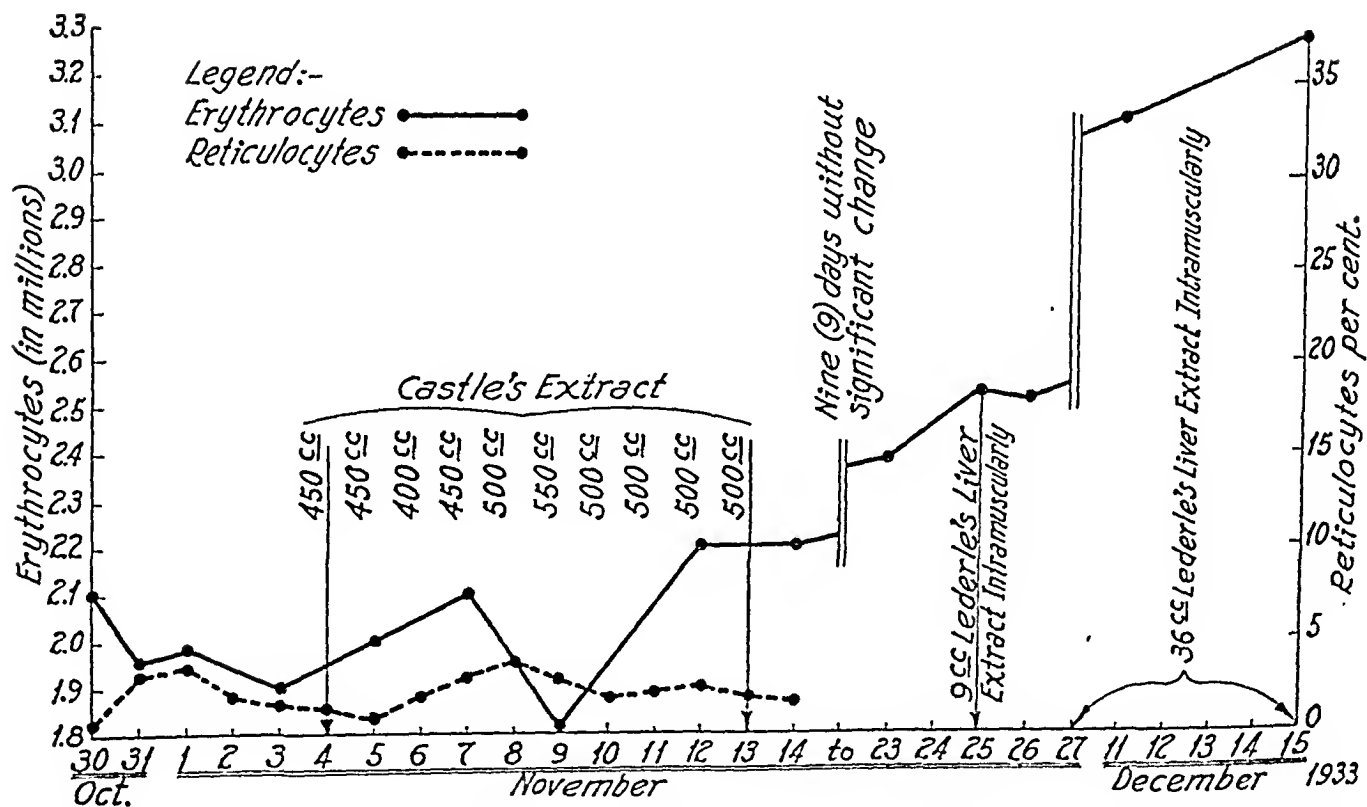


FIG. 5. CONTROL EXPERIMENT: NEGATIVE RESPONSE WITH EXTRACT OF DIGEST OF BEEF AND GASTRIC JUICE OBTAINED FROM A PATIENT WITH PERNICIOUS ANEMIA AFTER TREATMENT WITH LIVER EXTRACT. UNIT NUMBER 92478.

(Unit Number 62087), a female, age fifty-five, with classical pernicious anemia including combined cord degeneration. In this case the reticulocyte level was very low, less than one per cent, and the response reached a maximum of only 6.2 per cent, but the erythrocyte rise was marked, the count jumping from a level of approximately 1.8 million to 2.6 million two days after the last injection of Castle's extract. These two control experiments illustrate the positive type of response obtained with the extract of normal human gastric juice and beef.

Figure 5 illustrates a control experiment of a negative type. The gastric juice used in this instance was obtained from a patient with pernicious anemia who had been receiving parenteral liver extract therapy. The reticulocyte level remained relatively constant, varying from 0.5 per cent to 3.5 per cent before the period of administration of the extract and also during the period of its administration.

These three control experiments, all of which are in accord with the original observations of Castle, give us confidence in our technique and in attaching real significance to the negative results obtained with the gastric juice of the patient under discussion.

#### DISCUSSION

There are two phases of this subject to be considered: the clinical and the experimental. Combined cord degeneration with achlorhydria and without demonstrable abnormality of the blood picture over a period of three years is, in our opinion, sufficiently rare to require reporting. Anemia may appear in time, of course, and thus prove conclusively that the case is merely one of pernicious anemia in which the achlorhydria and the neurologic manifestations have long preceded the alterations in the peripheral blood. This would be in accord with the prevailing clinical opinion. It is noteworthy, however, that in very few of the cases of this type reported in the literature are adequate blood studies described during the period in which anemia is said to have been absent. Such studies are essential for they may disclose a slight but significant anemia or a definite macrocytosis. No such findings were obtained in this patient. In most of the reported cases (6, 12, 13, 11, 15, 17, 27, 28) the termina-

tion of the disease is not stated, but there are on record two instances of combined cord degeneration without anemia and with a fatal termination. It is possible that with a more critical attitude toward this group, more instances of combined cord degeneration with achlorhydria but without anemia and without "ergotism, pellagra, syphilis, arteriosclerosis and cachectic states" may be encountered and perhaps found to constitute a disease entity entirely unrelated to pernicious anemia.

The spontaneous remission of symptoms in this case is noteworthy. The objective neurologic findings, of course, did not regress materially; but, on the other hand, they showed no evidence of progression. This is as inexplicable at present as is the cause of the disease itself. If iron or liver had been administered enthusiastically, the failure of the disease to progress would undoubtedly have been considered as evidence of the value of the therapy (17, 27). In this statement we do not imply that liver therapy is of no value in the combined system disease of pernicious anemia. Our opinion is quite to the contrary although the subject seems to be a controversial one still (7, 22, 23, 24, 25). The number of cases of progressive neurological change in patients under adequate liver treatment is certainly small as compared with those seen in the pre-liver era; in fact, they are sufficiently rare to raise the question of the adequacy of the treatment in each instance. In the case under consideration, however, the situation is quite different and in our opinion liver therapy is not indicated at present because the diagnosis of pernicious anemia is, we feel, not established.

The experimental phase of this paper has to do with the interrelationships of the "intrinsic factor" of Castle, achlorhydria, pernicious anemia, and combined cord degeneration. We must confess that there are many aspects of the general problem which are not clear to us in spite of the excellent work of Castle and of other investigators in recent years (*supra* vide and 26). In this particular case we are quite unable to explain to our own satisfaction the absence of anemia. It is possible to assume that "intrinsic factor" is present in the gastric secretion in subminimal amounts which we failed to detect, but which were adequate to prevent the development of anemia.

This would be in accord with the findings of Isaacs, Goldhamer, and Sturgis (18). Exact methods for measuring quantitatively the amount of "intrinsic factor" are not available. The amounts of gastric secretion obtained and used by us, however, are comparable to those used by Castle and his coworkers and upon which they based their conclusion with regard to the absence of intrinsic factor in pernicious anemia. The quantities used by us were as great, in fact, as Castle, Heath and Strauss (11) obtained in simple achlorhydria and in sprue, and which they found adequate for the demonstration of "intrinsic factor." Consequently, our failure to obtain a positive response can be compared, we feel, to Castle's failure to obtain a positive response in pernicious anemia. It is possible, as the work of Isaacs, Goldhamer, and Sturgis suggests that the "intrinsic factor" is nevertheless present in minimal amounts. If it be argued that the quantity present in pernicious anemia is not adequate to prevent the appearance of anemia, it is difficult to understand why, in the case under discussion, anemia did not result from a similar decrease in the amount of intrinsic factor. The hypothesis might be advanced that our case contained a greater although not detectable amount of intrinsic factor than is present in pernicious anemia, but there is at present no evidence for this, unless one reasons in a circle and interprets the absence of anemia as evidence of a greater content of intrinsic factor. The fact remains that we failed to demonstrate the presence of intrinsic factor alike in our case of combined cord degeneration without anemia and a case of pernicious anemia under treatment.

It might be argued that anemia did not appear in our case because of the megalocytosis and consequent more complete absorption of small amounts of hematopoietic substance. This hypothesis is difficult to prove or disprove. It may be noted, however, that megalocytosis is not uncommon in tropical and non-tropical sprue and in celiac disease (30), and that in these conditions it does not prevent the development of a macrocytic anemia which is cured by the administration of liver (31).

The cause of the combined cord degeneration remains obscure. It is tempting to hypothecate, as have others, an "extrinsic factor" different from the anti-anemic extrinsic factor, interacting

with the same or another "intrinsic factor." These are interesting speculations, but more facts must be established before a definite theory can be formulated.

#### SUMMARY

1. An untreated case of subacute combined cord degeneration of three years' duration, without anemia, with achlorhydria, and with a prolonged spontaneous remission of symptoms is reported.

2. Two experiments designed to demonstrate the presence or absence of the anti-anemic "intrinsic factor" of Castle in the gastric juice of this patient are considered to have given negative results.

3. Control experiments are cited, showing positive results with normal human gastric juice and a negative result in pernicious anemia, all in accord with the observations of Castle and his coworkers.

4. We are unable to offer a completely satisfactory explanation for the absence of anemia, for the presence of the combined cord degeneration, or for the failure of the neurological process to progress during the past two years.

5. Our observations may be interpreted as in accord with either of two views: (a) that combined cord degeneration may be a disease *sui generis*, or (b) that it is invariably a manifestation of the same basic disorder as that which causes pernicious anemia.

#### Addendum

Since the preparation of this manuscript, the work of Greenspon (33) appeared apparently modifying the theory of Castle. It does not materially affect the concept with which this paper is concerned, i.e., the intrinsic factor, but it seemed worth while, nevertheless, to determine the pepsin content of the gastric juice of the patient described. A twenty-cubic centimeter sample was collected during a sixty-minute period following the subcutaneous injection of 0.5 mgm. of histamine hydrochloride. Miss Jean Hudson of Dr. Lester Dragstedt's laboratory, using the viscosimeter method (34), was not able to detect any trace of pepsin in the gastric juice. This would seem to substantiate further the validity of our work.

The patient continues to improve slowly symptomatically. The erythrocyte count March 25, 1936, is 4.21 million, hemoglobin, 82 per cent (Dare), leukocytes, 10,500. The gastroscopic picture as observed the same day remains unchanged, there being a spectacular atrophy of the entire gastric mucous membrane.

The very important work of Dickey and McKinley (35) and of Salus and Weiman (36) was not discovered unfortunately until after this paper had gone to press. The observations reported by these investigators, leading as they do to rather contradictory views, emphasize further the questions we have raised.

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# UNDERNUTRITION IN THE TREATMENT OF CORONARY ARTERY DISEASE (PARTICULARLY THROMBOSIS). EFFECT ON THE BASAL METABOLISM AND CIRCULATION<sup>1</sup>

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A low calorie diet in the treatment of coronary thrombosis has been systematically used by one of us for a number of years with good results (1). Assuming that the observations made by Benedict et al. (2), Lusk (3) and Du Bois (4) in normal people were applicable to those with acute heart disease it was thought that the beneficial results obtained could be attributed in part to the lowering of the basal metabolic rate and decrease in the work of the heart which such a diet would effect. An intensive investigation was therefore undertaken two years ago, and the results in eight patients have already been reported (5). We have now extended our studies to a larger series and are presenting our findings in forty-two patients (Table I).

## METHOD

Twenty-nine of the patients in this series were in the hospital wards with acute coronary artery thrombosis. The remainder were ambulatory and suffering from angina pectoris with or without a history of previous coronary occlusion. The former were kept in bed for four to six weeks, longer if necessary. All were placed on a diet of approximately 800 calories (1) consisting of 80 grams of carbohydrate, 50 grams of protein and 30 grams of fat. This diet is well balanced and calculated to supply an adequate amount of vitamins and minerals.

Fluids were moderately restricted, 1200 to 1500 cc. being allowed unless heart failure was present. Only in the latter condition was salt limited.

The low calorie diet was always maintained beyond the acute phase of the thrombosis, usually three months. The patient was then given graduated diets of 1200, 1500 and 2000 or more calories for adequate periods of time. Following

any of these periods the diet was often again reduced to 800 calories for comparison with the initial period.

To determine the effect of the 800 calorie diet on the basal metabolic rate, it was essential to have, as a control, accurate figures while the patient was on a regular diet. We attempted to obtain these by taking readings soon after admission to the hospital but when, as frequently happened, the seriousness of the conditions made this impossible, the control figures were determined after a regular diet had been resumed. In thirteen patients "normal" figures were obtained both at the beginning and at the end of the experiment, the results coinciding. With the ambulatory patients, control readings were usually obtained before the 800 calorie diet was instituted and while the patient was still on his regular diet.

## RESULTS

A drop of 15 per cent or more in basal metabolic rate was considered a significant effect of the low calorie diet on basal metabolism. Of the 42 cases studied, this result was obtained in 31 (74 per cent) and these we have designated successes. Six were only partially successful, that is, the basal metabolic rate fell 10 to 14 per cent. In five, considered failures, the drop was less than 10 per cent. Our results are recorded completely in Table I, which is arranged according to initial weight and includes age, sex, height, weight at the onset and the end of the diet, ideal weight,<sup>2</sup> basal metabolic rate and oxygen consumption at various levels of diet. In addition, seven cases have been chosen for graphic presentation (Figures 1 to 8).

<sup>2</sup> As obtained from Medico-Actuarial Mortality Investigation, Vol. 1, 1912, New York Association of Life Insurance Medical Directors and Actuarial Society of America.

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TABLE I  
Correlation between the caloric intake, weight and basal metabolic rate of 42 patients suffering from coronary artery disease

Case number	Hospital number	Sex	Age	Ideal weight	Initial weight	Height	Diet	Period on diet	Weight at end of period	Average O <sub>2</sub> consumption	Average basal metabolic rate	Days to attain average basal metabolic rate	Percentage drop in weight to attain low average basal metabolic rate	Percentage drop in total O <sub>2</sub> consumption	Percentage drop in basal metabolic rate	Remarks
			years	pounds	pounds	inches	calories per day	day	pounds	cc. per minute	per cent		per cent	per cent	per cent	
1.	377099	F.	60	131	108	59	Control 800 1600	1-27 27-230	108 100 105	174 137 165	+4 -16 0	12	3	21	20	Coronary thrombosis In bed Ambulatory
2.	374672	F.	57	141	118	63	Control 800 1200	1-82 82-174	118 103 103	165 127 137	-8 -27 -20	35 12	9	23 17	19 12	Coronary thrombosis In bed, day 1 to 36 Ambulatory
3.	376700	M.	45	138	118	62½	Control 800	1-112	118 100	205 184	+2 -5	13	7	10	7	Angina pectoris Ambulatory
4.	370703	M.	47	140	118	64½	1200 800 1600	1-100 100-121 121-271	107 104 114	173 154 173	-12 -21 -11	14 11	2	11	10	In bed, day 1 to 40. Coronary thrombosis Ambulatory
5.	381087	F.	61	138	140	62	Control 800 1500 Control 800	1-69 69-140 1-30	140 126 128 131 132 124	177 168 181 171 163	-6 -9 -3 -8 -10		4½	7 5	6 2	Coronary thrombosis, cyanosis In bed, day 1 to 50 Ambulatory
6.	382712	F.	60	141	142	63	Control 800 1500	1-16 60-123	142 134 132	209 180 206	+9 -4 +13	38	5½	14	15	Coronary thrombosis In bed Ambulatory
7.	31-18000	F.	50	133	140	60	Regular 1200	1-32	140 143	215 183	+11 -5	13	2	15	10	Angina pectoris Ambulatory
8.	373329	F.	44	141	150	64	800 1100 1500 1800 92-145 145-357	1-58 58-75 75-92 92-145 145-357	136 137 137 139 133	160 158 175 200 183	-21 -22 -15 -1 -8	16 9 27	4½	20 21 13	20 21 14	In bed, day 1 to 50 Coronary thrombosis Ambulatory
9.	383638	M.	45	135	130	61	Control 800 1200	1-41 41-77	130 123 124	196 155 170	-3 -23 -15	33 7	5½	21	20	Coronary thrombosis In bed, day 1 to 20 Ambulatory
10.	384231	M.	35	132	135	62	Control 800	1-32	135 116	213 198	0 -5	12	8	7	5	In bed. Coronary thrombosis
11.	374120	M.	43	146	140	65	800 1500	1-50 50-110	127 122	194 204	-10 -2	45	7	6	8	In bed. Coronary thrombosis, upper respiratory infection Ambulatory
12.	387039	M.	47	130	111	61	Control 800 1500	1-56 56-200	141 125 131	218 181 209	+1 -10 +2	41	11	12	11	Coronary thrombosis In bed, day 1 to 25 Ambulatory, working
13.	374016	M.	62	147	142	64½	800 1000 1200 1400 142-288 288-336	1-50 50-100 100-142 140-142 142-288 288-336	132 130 130 130 135 133	167 172 170 183 207	-18 -16 -17 -12 0	24	7	19 17 18 12	18 16 17 2	In bed. Coronary thrombosis Ambulatory Working
14.	35-1856	M.	38	144	145	65	1500 800 1500	1-50 56-93 93-127	145 137 139	212 176 207	-8 -23 -8	14 28	5	17	15	Coronary thrombosis In bed Ambulatory

TABLE I (Continued)

15.	372020	M.	50	150	145	03	800 1500 800 2000 1500 2000	1-30 30-47 47-61 61-113 113-328 328-370	120 130 131 141 108 142 138	-18 -13 -30 -5 -27 -10	17 4 12 27 7	0	18.5	13	In bed, Coronary thrombo- sclerosis Ambulatory Ambulatory
10.	31-4001	M.	55	154	147	00	Control 800 1200 1500	1-32 32-130 130-171 171-280	147 131 137 137	-4 -10 -31 -0	19	1	10 18	15 17	Ambulatory, angina pectoris Ambulatory Ambulatory
17.	381001	M.	40	151	148	00½	Control 800 1500	1-60 60-110 110-127	148 130 140 143	-0 -31 -5 -14	22 46 10	11	31	20	Coronary thrombo- sclerosis In bed Ambulatory
18.	35-10700	F.	45	132	151	01	Control 800 1200	1-77 77-69	151 131 131	-0 -17 -7	7	2	17	11	Ambulatory, angina pectoris Ambulatory
19.	33-11370	F.	51	133	151	00	Regular 1200	1-32	151 150	+13 -3	32	2½	10	10	Ambulatory, angina pectoris Ambulatory
20.	33-7157	F.	50	138	163	02	Regular 800 1500 1000	1-51 51-127 127-152	163 118 150 147	0 -15 +4 -11	12 30 15	0 2	17 15	15 15	Ambulatory, angina pectoris Ambulatory Ambulatory
21.	372310	M.	52	181	155	71½	Control 800 2000	1-30 30-78	155 147 152	+8 -10 +4	25 24	5	27	25	In bed, Coronary thrombo- sclerosis In bed In bed
22.	370117	M.	30	110	150	01	Regular 800 1000 1200 1500	1-7 8-80 80-150 150-200 200-300	150 142 110 140 155	+4 -15 -18 -18 -10	13	0	21 23 23 11	10 22 22 11	Ambulatory, Coronary thrombo- sclerosis In bed Ambulatory Ambulatory
23.	381101	M.	55	158	157	07	Control 800 1200	1-110 110-112	157 110 118	+14 -8 -13	27	7	21 25	22 37	In bed, day 1 to 40, Coronary thrombo- sclerosis
24.	381003	M.	43	155	160	07	Control 800	1-30	160 150	-5 -22	30	0½	10	17	Coronary thrombo- sclerosis In bed
25.	35-2361	M.	63	115	175	01	Regular 800 1200 1000	1-80 80-101 101-235	175 157 163 150	-5 -27 -21	30 30	7	23 20	18 10	Ambulatory, angina pectoris Ambulatory
26.	35-10600	M.	30	132	101	07	Control 1200 1500	1-50 50-100	101 154 118	+22 +1 +0	28 14	7	10 14.5	18 13	Ambulatory, angina pectoris Ambulatory
27.	373103	M.	50	110	165	01½	800 2000 800	8-30 30-63 63-85	155 160 152	-10 +5 -25	10 20 11	0 3	25 30	21 30	In bed, Coronary thrombo- sclerosis Ambulatory
28.	370155	M.	57	153	170	00	Control 800 2500	1-00 00-312	170 140 170	+8 -0 +7	41	12	17	11	Coronary thrombo- sclerosis In bed Ambulatory
29.	35-2341	M.	51	157	170	07	800 1500 1500	1-110 110-161 161-250	157 110 112	-10 -11 -3	31	3	25	107	In bed, day 1 to 50, Coronary thrombo- sclerosis Ambulatory Ambulatory

TABLE I (Continued)

Case number	Sex	Age	Ideal weight	Height	Diet	Period on diet	Weight at end of period	Average O <sub>2</sub> consumption	Average basal metabolic rate	Days to attain average basal metabolic rate	Percentage drop in weights to attain low average basal metabolic rate	Percentage drop in total O <sub>2</sub> consumption	Percentage drop in basal metabolic rate	Remarks
		years	pounds	inches	calories per day	day	pounds	cc. per minute	per cent		per cent	per cent	per cent	
30.	387168	M.	34	63	800 1200 1500	1-60 7-140 140-250	155 210 210	196 210 210	-19 -9 -12	14	7½	20?	19?	In bed. Coronary thrombosis Ambulatory Coronary thrombosis In bed, day 1 to 30 Ambulatory
31.	375120	M.	42	68	Control 800 1200 1000	Control 1-124 124-171 171-200	173 144 144 141	235 186 189 189	-6 -22 -16 -19	64 14	12	21 15 19½	16 10 13	Coronary thrombosis In bed, day 1 to 50. Coronary thrombosis In bed, day 90 to 120 Ambulatory Ambulatory In bed. Angina pectoris
32.	378312	M.	51	70	800 1000 1200 800 1500	1-205 205-238 238-267 267-276 270-351	140 142 141 138 140	150 180 168 150 180	-34 -31 -27 -34 -21	9 12		6	7	Ambulatory, angina pectoris
33.	367071	M.	54	68	1600 800	1-30 30-58	182 173	223 200	-11 -18	25	5 2	14 15	13 15	Ambulatory, angina pectoris
34.	31-11123	M.	52	68	1200 800 1800	1-20 20-55 55-151	180 176 181	210 209 243	-16 -18 -3		8	15	11	Ambulatory, working, angina pectoris Ambulatory
35.	35-601	M.	57	59	Control 800	Control 1-75	182 142	277 235	+22 +11	23				In bed, day 1 to 42. Coronary thrombosis Ambulatory, working
36.	378358	M.	56	68	Control 1000	Control 1-213	185 155	242 185	-21	0	10	23	21	In bed, day 1 to 42. Coronary thrombosis Ambulatory, working
37.	378530	M.	70	190	800 1200 1500	1-70 70-162 162-266	171 172 179	178 170 198	-19 -18 -12	14	5	20?	19?	In bed, day 1 to 40. Coronary thrombosis Ambulatory, working
38.	380015	M.	68	190	800 2000	1-38 38-176	177 183	210 228	-9 -2	20	5	8	7	In bed. Coronary thrombosis Ambulatory, working
39.	375512	M.	50	200	Control 800 1200 1500 2000 1800	Control 1-102 102-116 116-173 173-272 281-350 350-359 359-377	200 170 165 162 143 147 148 147	240 190 218 200 200 236 220	-5 -20 -6 -12 -5 +5 -1	24 36 40 14 7 14	4	21 9 17 13	15 1 10 0	Ambulatory, angina pectoris Ambulatory
40.	35-1582	M.	55	221	67 Control 800 1500 to 2000 800	Control 1-79 79-134 134-153	221 179 203 197	310 296 316 254	+16 +14 +23 0		11	4	2	Ambulatory, angina pectoris Ambulatory
41.	35-373	F.	53	222	61 Control 800	Control 1-110	222 171	214 175	-8 -21	76	14	18	13	Ambulatory, angina pectoris Ambulatory
42.	31-19036	M.	62	231	67 Control 800 1000 1400 2000 254-358	Control 4-120 120-150 150-185 185-254 254-358	231 179 178 180 188½ 178	253 187 238 202	-3 -22 -3 -16	30	9½	20	19	Ambulatory, angina pectoris Ambulatory
											27	15	13	Regular indicates 2500 calories.

It will be seen that the control basal metabolic rate varied between  $+10$  and  $-10$  in 83 per cent of the cases. The average in the different weight groups did not vary essentially; neither leanness

nor obesity was associated with a characteristic basal metabolism (6).

The drop in metabolism on the 800 calorie diet was sufficient to permit maintenance of the pa-

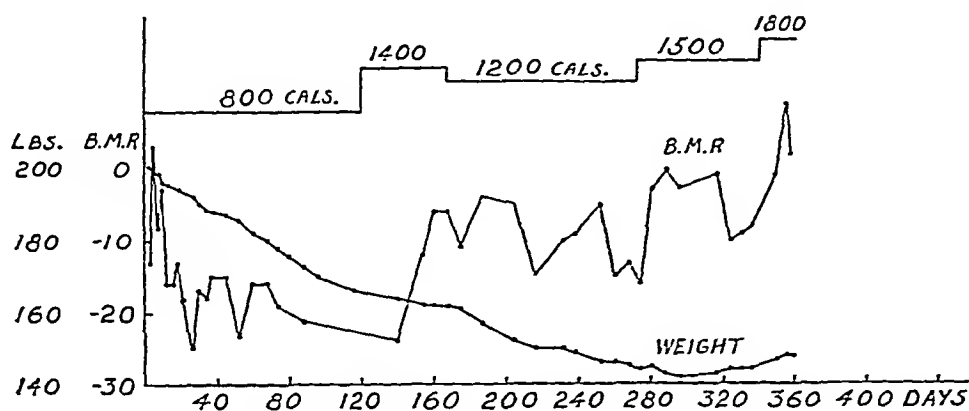


FIG. 1. CORRELATION BETWEEN DIET, WEIGHT AND BASAL METABOLIC RATE IN CASE 39, MALE, AGE 50.

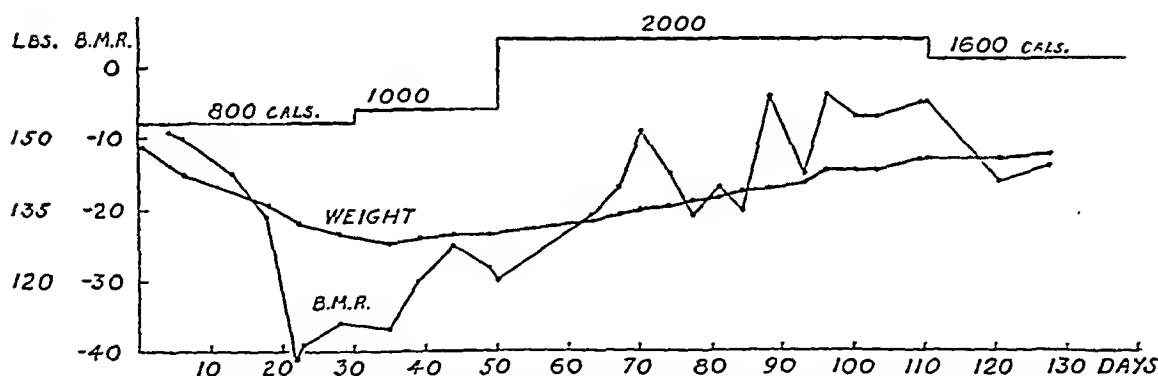


FIG. 2. CORRELATION BETWEEN DIET, WEIGHT AND BASAL METABOLISM IN CASE 17, MALE, AGE 40.

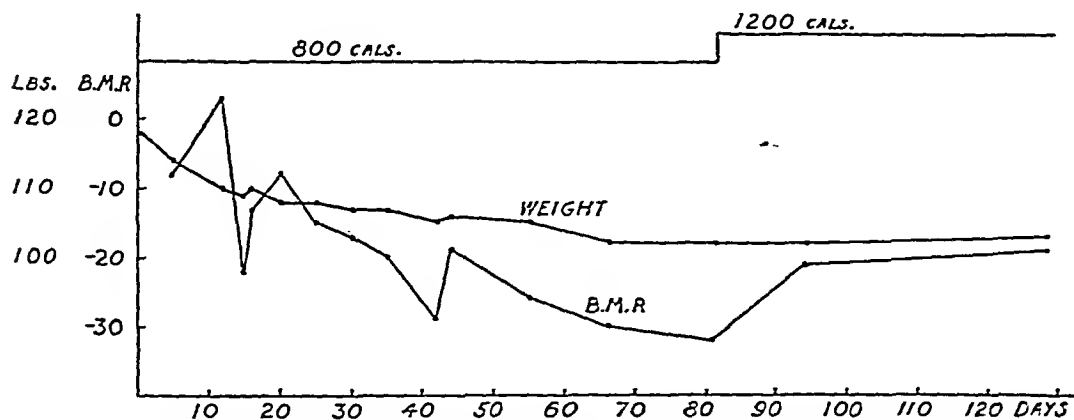


FIG. 3. CORRELATION BETWEEN DIET, WEIGHT AND BASAL METABOLISM IN CASE 2, FEMALE, AGE 57.

tients at a basal metabolic level of —20 per cent, or lower. In 22 of the cases the average drop in the basal metabolic rate was 15 to 20 per cent; in 9 it was 21 to 35 per cent. In the latter group a rate of —40 was reached in 2 cases, and —30 in 2 others. The fall in basal metabolic rate was of course found to parallel that in oxygen consumption. However, when a considerable loss in

weight occurred, because of the changes in body surface, the basal metabolic rate did not adequately reflect the actual drop in basal metabolism. Thus in Case 31, after a loss of twenty-nine pounds, or 17 per cent of the initial body weight, a drop of 21 per cent in oxygen consumption was equivalent to a fall of only 16 per cent in basal metabolic rate.

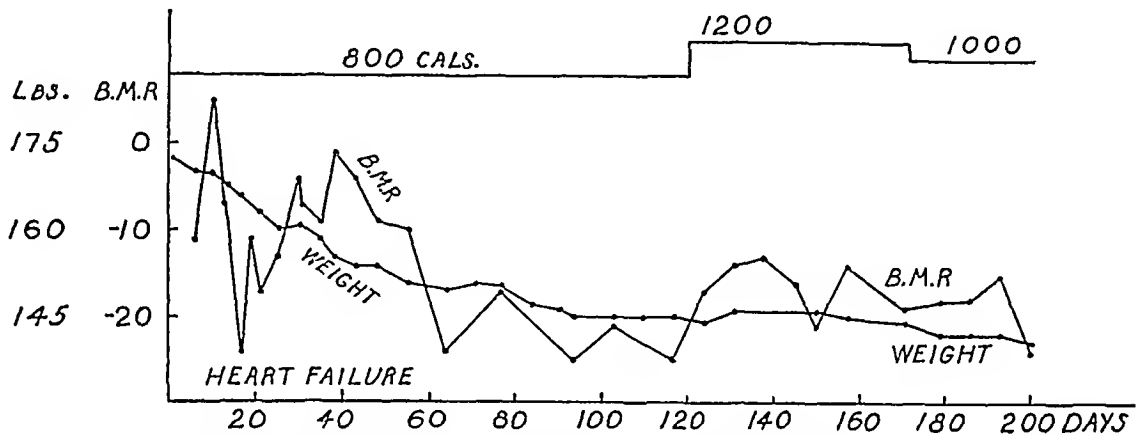


FIG. 4. CORRELATION BETWEEN DIET, WEIGHT AND BASAL METABOLISM IN CASE 31, MALE, AGE 42.

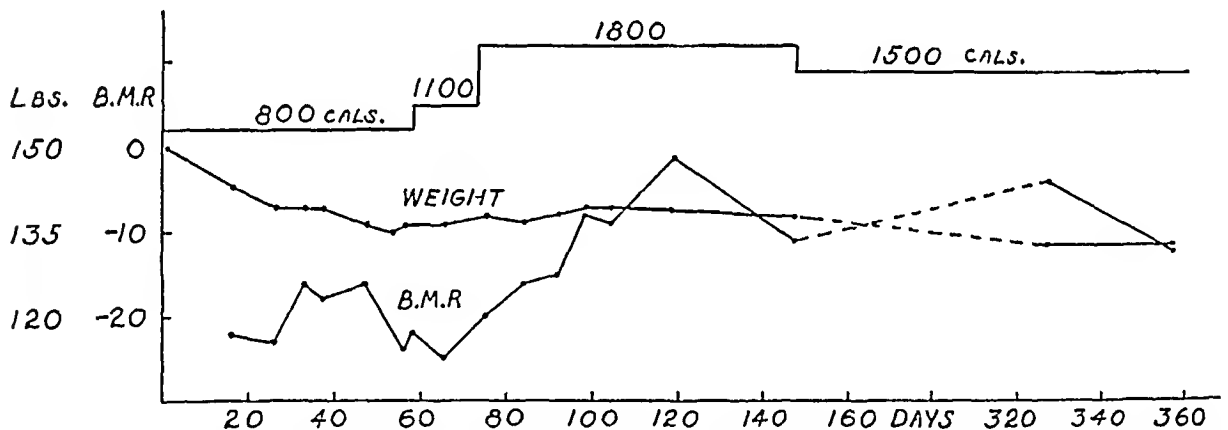


FIG. 5. CORRELATION BETWEEN DIET, WEIGHT AND BASAL METABOLISM IN CASE 8, FEMALE, AGE 44.

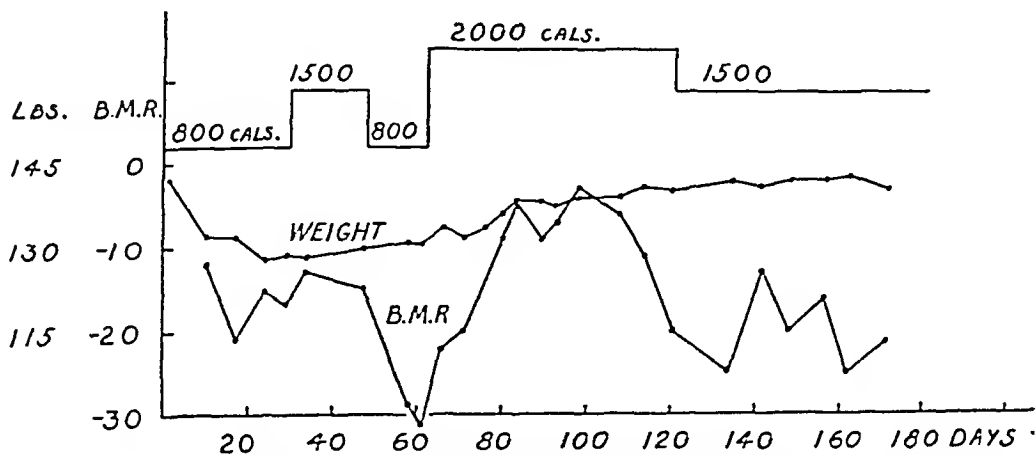


FIG. 6. CORRELATION BETWEEN DIET, WEIGHT AND BASAL METABOLISM IN CASE 15, MALE, AGE 59.

### *Time of drop in basal metabolic rate*

Following the institution of an 800 calorie diet the basal metabolic rate reached the low levels in an average of two to four weeks. When the diet was increased a rise in the rate did not occur until a similar period had elapsed. Despite a marked change in diet, therefore, the metabolism of the body is determined for several weeks by the previ-

given an 800 calorie diet, and the basal metabolic rates dropped to their previous low levels or even lower, and usually more rapidly than in the first instance. For example, in Case 15 (Figure 6), the basal metabolic rate on an 800 calorie diet fell to -21 per cent on the seventeenth day. On a 1500 calorie diet it rose to -7 per cent in ten days. When an 800 calorie diet was resumed, the

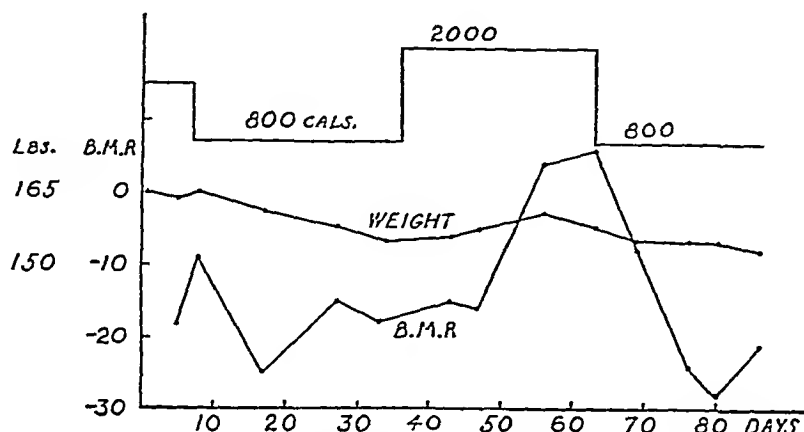


FIG. 7. CORRELATION BETWEEN DIET, WEIGHT AND BASAL METABOLISM IN CASE 27, MALE, AGE 50.

ous state of nutrition. In other words, if an 800 calorie diet has been taken for some time, a sudden increase even to 3000 calories daily for several days, will not influence the basal metabolism. Anderson and Lusk (7) reported similar findings.

A change in caloric intake eventually was associated with a distinctive change in basal metabolic rate. Certain levels of basal metabolism were usually established by varying caloric intakes, i.e., 800, 1200, 1500 or 2000. Thus if the basal metabolic rate had fallen to -20 on an 800 calorie diet (Case 39, Figure 1), it rose to -12 on a 1200 calorie diet, and to -6 on a 1500 calorie diet and on a 2000 calorie diet became +5. (See Figures 1 to 7 and Table I.) Not infrequently, there was no difference in effect between an 800 and a 1200 calorie diet (Case 16 and 32), and it was only after a regular diet had been resumed (2000 calories or more) that the basal metabolic rate returned to normal.

### *Repeated drops in basal metabolic rate*

Following the return to a regular diet several patients (Cases 15, 20, 27, 39, 42) were again

rate fell to -29 per cent, in eleven days. This more profound and more rapid effect was probably the result not only of the improved clinical condition of the patient but also of the previous undernutrition with the consequent depletion of excess body fat.

### *Factor of weight*

The drop in basal metabolic rate on the low calorie diet is obviously closely related to loss of weight. Indeed it might be expected that the decrease in basal rate merely reflected the degree and course of the weight loss. Richardson and Mason (8) observed that in diabetics treated with a low calorie diet the greater the loss of weight the lower the basal metabolic rate. However, our studies revealed some interesting deviations from this view.

The relation of loss of weight to drop in basal metabolic rate for various weight groups is presented in Table II. It will be seen that the results were fairly uniform for all weights below 200 pounds: the average drop in basal metabolic rate was 15.5 to 17 per cent; the loss of weight neces-



TABLE II  
Correlation of initial and ideal weight with loss of weight and fall in basal metabolic rate

Initial weight	Number of cases	Number of successful results		Percentage loss of initial weight necessary to obtain low basal metabolic rate level		Percentage drop in average basal metabolic rate	
				Average	Range	Average	Range
10 to 20 per cent below ideal weight. Average 123 lbs. Within 10 per cent below or above ideal weight. Average 149 lbs.....	5	3	60	6.0	3 to 9	17	10 to 24
10 to 35 per cent above ideal weight. Average 174 lbs.	19	15	79	6.5	1 to 11	17	5 to 34
35 to 65 per cent above ideal weight. Average 214 lbs.	14	10	71.5	5.5	2 to 12	15.5	7 to 24
	4	2	50	11.0	9.5 to 14	16.5	11 to 23

sary to attain this drop was almost constant, averaging 6 per cent of the initial body weight. The average loss for the entire period of the diet was 9 to 13 per cent but it must be remembered that the actual loss of weight in pounds was greater in heavier patients. It is apparent that the percentage drop in weight was less than that in the basal metabolic rate, that is, a 6 per cent loss of weight was associated with a drop of 20 per cent in basal metabolic rate.

The loss of weight usually preceded the drop in basal metabolic rate, but in a few cases (e.g., Case 16) even when the patients had been on the diet for some time, the basal metabolic rate fell although little or no weight loss had occurred. This sequence was found especially in patients who were placed on an 800 calorie diet for a second period and is well illustrated by Case 15 (Figure 6). When this patient returned to an 800 calorie diet the basal metabolic rate dropped rapidly to —31 per cent without any loss of weight.

A study of the course of weight loss in our patients is of considerable interest. On this basis they fell into several groups. Most commonly there was a progressive loss of weight from the beginning to the end of the diet (Figure 1). In another large group which included the cases of cardiac failure, the loss in the first week or two was rapid and then became slowly progressive as in the first group (Figures 2, 3, 4, and 6). In several patients, loss of weight ceased after a number of months, although the diet was continued (Figures 2 and 4). Finally, a few pa-

tients began to lose weight only after the diet had been in effect one or two weeks. Similarly, when a regular diet was resumed most of the patients gained weight progressively, although in some a lag of one or two weeks was observed (Figure 5 and Case 4, Table I).

### Water metabolism

These variations have been shown to be closely related to the water metabolism of the body (9, 10). The initial loss of weight, usually rapid, is largely the result of a depletion of fluids, as well as of tissue. Later the weight loss, dependent chiefly on depletion of the body tissues, diminishes progressively. On the other hand, the failure to lose weight early, displayed in some cases, is best explained by water retention, a fact that has been emphasized by Newburgh and Johnson (10).

### Factor of obesity

In the three patients weighing 220 to 230 pounds (Cases 40, 41, 42, Table II), the total loss of weight as well as that required to produce a fall in metabolism was almost twice as great as in the less obese group; yet the average percentage drop in metabolism was the same. That is, it was not until 25 to 30 pounds had been lost that the basal metabolic rate began to drop. Only one case (Case 42) in which the patient's weight exceeded 180 pounds gave a reading below —20 per cent. It would seem that in the very obese, there is little need of conserving the excess weight by a reduction in basal metabolism.

### Ideal weight

It might be expected that the basal metabolism would drop only as the ideal weight was approached. In our cases, however, we found no correlation between the two (Table II). Eighteen patients were 10 per cent or more above their ideal weights; in twelve of these, the basal metabolic rate fell significantly long before the ideal weight was approached, particularly in the very obese. Furthermore, in those who eventually attained their ideal weights there was no additional reduction in the basal metabolic rate as the loss of weight continued. Secondly, in four of the group of nineteen patients whose ideal and actual weights approximated each other, the drop in ba-

sal metabolism was less than 15 per cent. Finally, of five patients 10 to 20 per cent below their ideal weights, the basal metabolic rate dropped less than 15 per cent in two. It is thus apparent that the reduction in basal metabolism occurs irrespective of the ideal weight.

#### *Food requirement for maintenance of weight*

In 14 cases, as a result of repeated changes in diet we were able to investigate a point of considerable theoretical importance, that is, the number of calories required to maintain weight in various stages of activity. Benedict et al. (2) and Muller (11) found that after a restricted diet a considerable diminution in food intake was required to accomplish this end. As a rule, with our patients only 1200 to 1500 calories were necessary to maintain weight when the patient was ambulatory. This subject, however, is being investigated completely, and the results will form a later report.

#### *Explanation of failure of basal metabolism to fall*

As was stated earlier, the basal metabolic rate dropped less than 15 per cent in eleven cases. In most of these a satisfactory explanation could easily be found for this. For instance, if an obese patient did not lose sufficient weight (Cases 33, 38, 40) the basal metabolic rate did not fall significantly. A second important factor proved to be cardiac failure. In other cases the lack of success could be attributed to fever and infection, particularly of the upper respiratory tract (Cases 11, 41); to frequent attacks of angina pectoris (Cases 3, 6, 33), and to abnormalities of the lungs, such as emphysema and bronchitis (Cases 35, 38). Several patients impressed us by their hyperthyroid habitus (Cases 3, 6, 10, 26), which perhaps helps to explain our inability to reduce their basal metabolic rates sufficiently.

#### *Effect of heart failure*

Although, as a rule, we began our readings only after heart failure had disappeared, in some cases we determined the basal metabolic rate in this condition (Cases 23, 31, 33). It was found elevated uniformly, but fell rapidly when the failure was relieved. Case 31, a patient subject to transient attacks of pulmonary edema, illustrates this point (Figure 4). There was little change in his

basal metabolic rate for sixty-four days, during which time cardiac failure was present; as this disappeared the basal metabolic rate dropped. Even in patients with heart failure, however, the low calorie diet may effect a drop in basal metabolic rate. For example, Case 23 (Table I) showed persistent moderate left heart failure with a few basal râles, prolonged circulation time and reduced vital capacity. For four weeks, his basal metabolic rate did not fall below zero; then, despite the persistence of the above findings, it gradually dropped from  $+10$  to  $-10$  per cent. In general, the lowest basal metabolic readings were obtained in patients without any evidence of heart failure.

There are numerous references (12) to dyspnea, tachycardia, diastolic hypertension and cyanosis in the literature as factors in heart failure which might be responsible for the elevated basal metabolic rate. Case 5 (Table I) is an example of the effect of cyanosis. Peabody, Meyer and Du Bois (12), Harrison (13), and recently Resnik and Friedman (14) have emphasized the increased muscular effort in dyspnea. The latter authors also pointed out the augmented oxygen consumption of the failing heart, which Starling and Visscher first showed experimentally (15).

#### *Possible ill effect of diet*

The possible ill effects of the prolonged undernutrition to which our patients were subjected were carefully sought for clinically and in the laboratory. We found none. There were no significant changes in the blood sugar which remained above 70 mgm. per 100 cc., and the serum protein was well within normal limits. Lusk (3) and Rubner (16) had already found a negligible loss of body protein on a diet similar to ours. Because of the intimate relation of the blood cholesterol and basal metabolism in myxedema (17), we made repeated determinations of the former in our patients. There was no definite change from the control figures, irrespective of the diet, drop in the basal metabolic rate and loss of weight. Nor was any alteration in cholesterol obtained by Poindexter and Bruger (18) who treated 30 obese subjects with a low calorie diet.

In no case was ketosis or dehydration en-

countered although the urine was examined frequently for acetone, and blood counts were done frequently. In fact, there was no demonstrable change for the worse in our patients even after long periods on an 800 to 1200 calorie diet. Eight patients remained on the diet for three to six months, 6 for six to nine months and 2 for nine to twelve months (Cases 39, 42).

#### *Effect of low diet on heart and circulation*

We were especially interested in determining the effect of the low calorie diet on the heart and circulation. An attempt was made to compare

TABLE III

*Effect of low calorie diet and low basal metabolic rate on basal pulse rate and blood pressure*

Case	Diet	B.M.R.	Pulse rate	Systolic blood pressure	Diastolic blood pressure
	calories	per cent	per minute	mm. Hg	mm. Hg
3	800 Regular*	-6 -10	52 58	98 to 108 146	58 to 66 82
6	800 1500	-5 +13	60 to 64 80	154 to 164 174 to 186	76 to 78 112 to 116
12	800 1500	-10 +2	55 to 62 62 to 70	130 136	72 78
13	800 1200 1500	-19 -20 -12	58 to 60 58 to 62 60 to 68	98 to 108 100 to 104 102 to 120	70 to 78 58 to 68 70 to 78
16	800 1500	-20 -11	58 to 63 54 to 60	126 to 134 145 to 150	80 to 82 90
17	800	-30 to 40 -4 to 7	54 to 60 70 to 80	96 to 110 126 to 140	64 to 78 86 to 90
18	800 Regular	-20 -5	48 to 58 73 to 80	114 to 126 134	76 to 82 92
19	1200 Regular	-3 +15	62 58 to 68	180 218 to 268	100 100 to 110
20	800 1500	-15 +4	55 to 60 68	148 to 158 170	80 to 100 90
22	800 Regular	-20 -1	58 to 60 64 to 68	92 to 104 112 to 122	66 to 70 80 to 88
25	800 1200 Regular	-24 -20 -5	48 43 70	124 120 to 130 130 to 140	76 78 80 to 90
26	1000 Regular	+4 +20	60 to 64 84 to 92	138 to 140 136 to 140	80 to 84 80 to 86
32	800 1500	-30 to 39 -10 to 26	48 to 52 54 to 62	98 to 106 90 to 112	70 to 84 60 to 75
39	800 1500 2000	-20 -9 0	44 to 46 48 to 56 60	130 to 146 146 to 162 160	90 to 96 84 to 90 90
40	800 Regular	0 +23	60 to 68 77 to 80	160 to 190 180 to 230	90 to 110 110 to 120
41	800 Regular	-21 -8	56 to 60 64 to 68	148 148 to 165	84 100 to 106
42	800 2000	-22 -3	52 to 56 56	136 to 156 154 to 176	80 to 90 94 to 100
Average	800 1500 to 2500	-16 0	56 68	132 152	81 90

\* Approximately 2500 calories.

the pulse rate and blood pressure at varying levels of caloric intake and basal metabolism. Obviously, in the cases which had suffered an acute thrombosis, with precipitate changes in the blood pressure and moderate alterations in the pulse rate, only those readings obtained several months after the acute episode had occurred should be considered. This difficulty does not arise in the ambulatory patients with angina pectoris. Seventeen patients were found suitable for such a study, the results of which are presented in Table III. All the readings were taken under basal conditions immediately after the determination of the basal metabolic rate.

It is noteworthy that even in these very seriously ill cardiac patients, the pulse rate on an 800 calorie diet was slow, usually between 50 and 60 beats per minute. In two cases (Cases 25, 39) it fell below 45. An increase in diet effected a rise in pulse rate in most cases. This is well illustrated by Cases 6, 17, 18, 19, 25, 26, 39, 40. It will be seen that on a regular diet the blood pressure also rose, both the systolic and diastolic pressures being effected. Since the elevation in the former was greater, the pulse pressure as a rule, was increased 10 to 20 mm. We are aware that some of these cases were suffering from essential hypertension in which variations in blood pressure may occur spontaneously. It also may be that the rise in blood pressure in the cases of coronary thrombosis was a delayed consequence of the acute episode. We wish to point out merely that in the cases accepted as suitable, the blood pressure had been stationary for several months on the low calorie diet, and only when the caloric intake was increased, did it rise, sometimes within a week.

#### *Effect of low diet on blood velocity and vital capacity*

The relation of changes in blood velocity and vital capacity to changes in diet and basal metabolic rate is also difficult to estimate in our cases for the reasons given above. However, by careful selection we were able to use eleven patients for such a study (Table IV). The blood velocity, as measured by the saccharin arm-to-tongue time (19), was determined in nine cases. In eight of these, it remained normal, that is 12

TABLE IV  
Correlation of basal metabolism, blood velocity and vital capacity

Case	Diet	B.M.R.	Circulation time	Vital capacity
	<i>calories</i>	<i>per cent</i>	<i>seconds</i>	<i>cc.</i>
4	800	-21		3000
	1600	-11		3000
8	800	-21	15	2100
	1800	-1		2000
13	800	-18	16	3000
	1800	0		2900
14	800	-23	12	
	1500	-8		
15	800	-20	21	
	1500	-20	20	
	1800	-10	17	3000
17	800	-34	15	4100
	2500	-5	15	4100
29	800	-19	16	3200
30	800	-19	16	3400
	1200	-9		3600
32	800	-34	16	3300
39	800	-20	16	3300
	1000	-15	18	3500
	1500	-5	14	3300
	1800	0	20	3300
42	800	-22		3300
	2000	-3		3100

to 16 seconds, when the basal metabolic rate had dropped during the period of the low calorie diet. In three of these cases, the readings were repeated when the basal metabolic rate returned to normal; there was no change in circulation time. Hence, it is seen that the blood velocity is perfectly normal in undernutrition in spite of the low basal metabolism. Macy, Claiborne and Hurxthal (20) have also found a normal blood velocity in other types of hypometabolism not associated with myxedema, for example, that seen in hypopituitarism.

The vital capacity was not effected by the lowered basal metabolism (Table IV).

We wish to point out that the majority of the patients considered "failures" or "partial successes" from the standpoint of drop in basal metabolic rate, improved clinically as did the successful cases. Table III shows that some of them also presented beneficial effects on the cardiovascular system. The loss of weight was as

great as in the successful cases. To this may be attributed in part the clinical improvement. It is possible that the basal metabolic readings obtained in these cases did not reflect the lowering of total energy expenditure actually present.

#### DISCUSSION

Our results indicate that patients with heart disease respond to a low calorie diet (800 calories) as do normal people, i.e. with a drop in basal metabolic rate of 15 to 35 per cent. Several possible criticisms of the validity of our figures must be discussed. Did the state of bed rest in ward patients influence the basal metabolic rate? In the cases of coronary thrombosis could the readings obtained early, while the patient was still suffering from the effects of the acute episode, though no longer acutely ill, be used as controls? Conversely, could the readings obtained after resumption of a regular diet be used as controls? Is an average drop of 15 per cent a significant fall in basal metabolic rate?

We do not believe that bed rest affected the basal metabolic rate appreciably since the low rates persisted after the patient became ambulatory. Also, the basal metabolic rate of patients in bed on a regular diet did not fall significantly.

As to "control" readings, it was often impossible at the beginning to determine the normal basal metabolic rate because of the condition of the patients. In these cases, however, control readings were obtained later when a regular diet was instituted. The control reading was practically the same in whatever period it was obtained. In 13 cases, controls were determined both at the beginning and end of the experiment and all agreed within 5 per cent which is within the limits of error of the method. It would seem then that since the control reading was practically constant, both the early and late readings represent the normal for the patient. Incidentally, the normal readings in most of our cases do not confirm an impression that the basal metabolism in coronary artery disease is low, for in only one case was the reading below -10 per cent.

Finally, we chose a reduction of 15 per cent in basal metabolic rate to designate a successful case, despite the fact that normally the basal metabolic rate lies between +10 to -10 per cent. This normal variation applies, of course, to a group of

people, whereas in the same person each of a series of readings under similar circumstances will vary little from the average of all readings. Since in this investigation we are dealing with changes in the average of many readings in the same patient, an average reduction of 10 per cent in basal metabolism may be really significant. However, we insisted upon a minimum of 15 per cent as evidence of a significant influence of the diet.

Although this is the first systematic study of patients with cardiac disease treated with a low calorie diet, the influence of undernutrition on the basal metabolism of normal people has been a subject of investigation for many years (21, 22, 23, 24, 25, 26).

Recently DuBois (4), cognizant of the beneficial effect of a low basal metabolism on the heart, expressed the hope that an agent capable of depressing metabolism would be introduced in the treatment of heart disease. A low calorie diet may satisfy this need. As far back as 1900, Hirschfeld (9) had been led by theoretical considerations to conclude that undernutrition lightened the work of the heart. Lusk (3) was aware of the influence of a low basal metabolic rate upon the cardiovascular system and referred to the conclusion of Determan (27) that the reduction of the burden upon the heart and blood vessels which occurs in undernutrition must be beneficial in heart disease. The Karell (28) diet consisting of 800 cc. milk daily, has been used in the treatment of cardiac failure for many years. The favorable results obtained with this diet may be attributed not only to the restriction of fluids but to the lowered caloric intake. In the recent German literature there are references (29) to the use of a low calorie diet and small meals in heart disease especially coronary artery disease.

Ample evidence is now available to prove that undernutrition decreases the work of the heart. The latter may be calculated from the formula  $W = VP + \frac{mv^2}{2g}$  (30), where  $V$  is the minute volume output of the heart and  $P$  is the mean arterial pressure.<sup>3</sup> The minute output is the

<sup>3</sup> The second half of the equation is negligible unless the velocity of the blood flow is unusually rapid;  $m$  is the mass of blood ejected per minute,  $v$  the velocity of blood flow and  $g$  the acceleration of gravity.

product of stroke volume and heart rate. Since the stroke volume is a function of the pulse pressure (31) and since the mean arterial pressure is practically the arithmetical mean of the systolic and diastolic blood pressures, it is evident that the work of the heart is dependent on the blood pressure and pulse rate. Slowing of the pulse and decrease in blood pressure and pulse pressure produce a reduction in cardiac work. This is exactly what happens in undernutrition. In Benedict's (2) cases on a 1400 calorie diet, the average pressure and pulse rate began to drop at the end of the first week. After three weeks the systolic blood pressure had fallen from an average of 120 to 94 mm., and the diastolic from 83 to 64 mm.; hence, the pulse pressure was diminished from 37 to 30. The pulse rate was slowed, in some cases, to 35. Incidentally, the electrocardiograms in these patients were normal. Rubner (16) also found a drop in pulse rate and therefore reduced cardiac work in undernourished persons. Our results show that the influence of a low calorie diet is exerted in patients with cardiac disease as in normal people, with a consequent reduction in the pulse rate and blood pressure.

Slowing of the pulse rate not only lessens the work of the heart but has been shown experimentally to be most efficient for the heart, since less oxygen per unit of time is required for a given amount of work (15). Furthermore, Benedict et al. (2) believed that the fall in pulse rate in his experiments indicated a minimum demand on metabolic activity and that a higher pulse rate in the same individual was usually associated with increased metabolism.

Definite proof of a decrease in the work of the heart as a result of reduction in the basal metabolic rate was recently provided by Altschule (32). Using the Grollman method, he found a pronounced reduction in cardiac output in patients subjected to total thyroidectomy. The cardiac output fell more rapidly than did the oxygen consumption. He concluded that a drop in basal metabolic rate of 30 per cent effected a reduction of 40 per cent in the work of the heart. A similar study was made in Case 17 of our series. The average basal metabolic rate when this patient was on the 800 calorie diet was — 34 per cent; the

cardiac output measured 2.76 liters per minute. When the diet was increased the basal metabolic rate rose to — 5 per cent and the cardiac output to 4.15 liters. Hence, the low calorie diet had caused a reduction of 33 per cent in cardiac output. The pulse rate which had averaged 58 on the low diet, rose to 71; the systolic blood pressure rose from 96 to 128 mm., and the diastolic from 65 to 86. Using the formula given above, it was calculated that there was a reduction of 49 per cent in the work of the heart during the low calorie intake.

The reduction in cardiac output in undernutrition is not associated with diminished cardiac efficiency. Benedict (2) studied his subjects carefully from this point of view and subjected them to graduated exercise tests. For short periods of work the percentage increase in pulse rate and blood pressure and also the time required for their return to resting levels, was similar to that for men on a regular diet; and even with more strenuous work there was no discernible modification of the functional efficiency of the heart. As early as 1903, Chittenden (33) studied the effect of excessive muscular exercise on a normal individual who subsisted on a diet of 1700 calories and found no evidence of mental or muscular inefficiency or strain on the heart and lungs. Similarly, Joffe, Poulton and Ryffel (34) by measuring the respiratory exchange after exercise observed no impairment in cardiac efficiency of their patient, a vegetarian who had been on a 300 to 500 calorie diet for three weeks and then on 1000 calories.

Our patients, too, after they recovered from the acute episode, returned to a state of moderate activity. Despite a continued low basal metabolic rate the vital capacity and exercise tolerance did not fall and occasionally improved. The circulation time did not change. Pain was minimal. The obese were distinctly benefited by the increased efficiency of the circulation. It is significant that the output and work of the heart were diminished without any slowing of the blood flow.

The foregoing considerations seem to indicate that a low food intake with its minimal demand on the heart is definitely beneficial. Therefore the use of the low calorie diet in acute heart disease seems logical.

It is interesting to consider the mechanism involved in the drop of basal metabolism in our patients. Lusk (3) had speculated about a diminu-

tion in thyroid secretion. However, it is apparent to us that the reduction in our patients was not mediated through the thyroid gland as there were no indications of hypothyroidism in the form of myxedema, rise in blood cholesterol (17) or delay in circulation time (35) even with a basal metabolic rate of — 30 or — 40 per cent. Indeed, these patients were as alert and efficient at this level as when on a regular diet with a normal basal metabolic rate. We believe with Benedict and Lusk, that the drop in basal metabolism is a protective adaptation of the body to the low calorie intake.

The low calorie diet treatment with the resultant drop in basal metabolic rate and decrease in work of the heart, naturally invites comparison with the procedure of total thyroidectomy which seeks the same objectives. Do we, by undernourishing our patients, attain the same results in the economy of the heart without the risk of a serious and difficult operation? It must be remembered, however, that we have been concerned chiefly with cases of coronary thrombosis, whereas total thyroidectomy (36) has been employed in patients with repeated cardiac failure or intractable angina pectoris. A longer study of our cases of angina pectoris is required before we can judge our results; with cases of persistent cardiac insufficiency we have had little experience. It is in the acute phase of heart disease, particularly coronary thrombosis, that our method seems most useful. On a low calorie diet some of our patients have been maintained at a level of — 20 for six to nine months. This is the level of basal metabolic rate the Boston group (37) has tried to attain by total thyroidectomy. The marked improvement in many of our most seriously ill patients, following the resumption of a diet sufficient to maintain weight, indicates that undernutrition for a period of months may improve the condition of the patient to such an extent that radical measures, such as total thyroidectomy will be unnecessary.

We are most grateful to Dr. Eugene F. DuBois for his keen interest and for his help and many suggestions. Dr. DuBois has encouraged us in this work for a long time.

#### SUMMARY AND CONCLUSIONS

1. The effect of a low calorie diet (800 calories) on basal metabolism was studied in twenty-

eight patients with coronary thrombosis and in fourteen with angina pectoris, whose control basal metabolic rate was within normal limits.

2. In thirty-one patients (74 per cent) the basal metabolic rate was lowered 15 to 35 per cent; such a drop was considered significant. In six patients, the basal metabolic rate fell 10 to 14 per cent, and in five, less than 10 per cent.

3. The time required for the basal metabolism to drop was two to four weeks. A similar period was required for its return to normal following the resumption of a regular diet. The body metabolism is determined for several weeks by the previous state of nutrition.

4. Following a period of undernutrition a subsequent period produces a more rapid and profound drop in basal metabolism.

5. The loss of weight necessary to attain a significant fall in basal metabolism averaged 6 per cent of the initial body weight.

6. The following factors tended to prevent a significant fall in basal metabolism; insufficient loss of weight, cardiac failure, upper respiratory infection, and repeated attacks of angina pectoris.

7. No ill effects resulted from the low metabolism induced by prolonged undernutrition of from 3 to 12 months duration. The blood cholesterol, sugar and protein were unaffected.

8. Graduated increases in diet to 1200, 1500 and 2000 calories often resulted in corresponding rises in basal metabolism.

9. The drop in basal metabolism is not accompanied by such evidences of hypothyroidism as myxedema, diminished blood velocity and hypercholesterolemia. Vital capacity is not affected.

10. The lowered basal metabolism had a beneficial effect on the cardiovascular system, resulting in slowing of the pulse rate, decrease in blood pressure and pulse pressure and diminution of the cardiac output and work of the heart.

11. A low calorie diet often relieves the symptoms of heart disease.

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## STUDIES OF ASCORBIC ACID AND RHEUMATIC FEVER

### I. QUANTITATIVE INDEX OF ASCORBIC ACID UTILIZATION IN HUMAN BEINGS AND ITS APPLICATION TO THE STUDY OF RHEUMATIC FEVER<sup>1</sup>

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In recent publications, Rinehart and Mettier (1933, 1934), and Rinehart, Connor and Mettier (1934) have offered evidence to the effect that chronic scurvy with superimposed infection in guinea pigs results in a histopathological picture "strikingly similar" to that found in rheumatic fever in human beings. Moreover, they have cited other experimental, epidemiological, and clinical data in favor of a theory that vitamin C deficiency may be a necessary accompaniment of the infection associated with this disease. Their experimental results with guinea pigs have, to some extent, been confirmed by Schultz (1936).

The experimental evidence in favor of this concept is inadequate because no one has yet succeeded in producing rheumatic fever experimentally in any species, and because two different species have been used in an attempt to detect a pathological similarity between two different diseases—scurvy, and rheumatic fever. Furthermore, the other factors tending to make apparent a relationship between latent scurvy and rheumatic fever possess the limitations imposed upon any evidence of a purely presumptive nature.

We have, therefore, endeavored to test this concept of Rinehart and his collaborators, by a direct study of the vitamin C<sup>2</sup> utilization in rheumatic and non-rheumatic patients. The present paper deals with the development of a method for the study of ascorbic acid nutrition in individuals,

upon the basis of which, a quantitative criterion of adequacy or deficiency with respect to this substance has been established. With this index of nutrition as a base-line, a comparative study of rheumatic fever subjects and suitable controls has been made.

Eekelen, Emmerie, Josephy, and Wolff (1933) were the first to state that vitamin C could be detected in blood and urine. At about the same time, Harris, Ray and Ward (1933) independently demonstrated a correlation between the urinary excretion of ascorbic acid and the dietary intake, and suggested that their technique could be used for the diagnosis of hypovitaminosis-C in human beings. They administered a single large dose of orange juice to normal individuals and obtained an immediate marked rise in the daily urinary output of ascorbic acid, the level of which dropped off almost to normal on the following days when no dose was taken. Individuals previously on normal, adequate, diets excreted ascorbic acid at a steady rate even after the material was omitted from the diet for several days, thus indicating that a store of the substance was present in the body.

Hess and Benjamin (1934), studying presumably normal infants, found no appreciable amount of ascorbic acid in normally excreted urine, nor did they obtain the marked rise after feeding found by Harris, Ray, and Ward (1933), even after a daily dose of one pint of orange juice had been added to the diet. Although they themselves did not emphasize the difference between the reactions of infants and adults, it is apparent that their results are in agreement with those of the latter workers, in that the urinary output or response to a given dose, is a function of the body storage or degree of "saturation" which in turn is dependent upon the previous dietetic history and the demands of the organism. It is, therefore, probable that growing children require

<sup>1</sup> Presented in preliminary form, at the meeting of the American Society for Clinical Investigation, Atlantic City, N. J., May 6, 1935.

<sup>2</sup> The antiscorbutic substance, initially named vitamin C, has also been called hexuronic acid, ascorbic acid, cevitic acid, "cebion," "cevit," and "redoxon." However, the term used by those who did the pioneer work in the identification of the substance, the elucidation of its structure, and finally, its synthesis, and the one now generally accepted by chemists, is ascorbic acid. We shall, therefore, as a rule, refer to it hereafter, by that name.

larger amounts in proportion to their size, than do adults.

Johnson and Zilva (1934) strengthened the idea that the daily urinary excretion of ascorbic acid is dependent not only upon the dose, but that the response to any given dose is a function of the amount stored in the body. Using orange or lemon juice, they found that for any adult the final level of urinary output attained (at "saturation"), is approximately constant, and proportional to the daily intake. However, the *percentage* excretion of ascorbic acid decreases with increase in dose.

More recently, Harris and Ray (1935) have applied the method of Harris, Ray and Ward (1933) to the detection of ascorbic acid deficiency in scorbutic infants and in other subjects ranging in age from eight months to eleven years. They found in scorbutic children that the low daily urinary output was increased to normal after cure. Furthermore, the feeding of large test doses of 100 mgm. of pure ascorbic acid to such patients induced responses entirely different, before and after cure. In the case of an adult (age not given), on an ascorbic acid-free diet, the addition of a daily intake of 220 cc. of orange juice, equivalent to 140 mgm. of ascorbic acid, resulted in only a slightly increased excretion over the amount previously excreted. When 280 mgm. of the pure substance were given daily (in four doses), however, the individual became "saturated" in 6 days, and finally excreted 225 mgm. daily, or 80 per cent of the intake. When the ascorbic acid was discontinued, the urinary output rapidly fell again. Harris and Ray also found that the ascorbic acid content of the previous diet had the same effect on the results of the Göthlin (1931) capillary permeability test, as on the urinary output results. In their conclusions, they suggested that test doses be graded according to the body weight of the subject.

It appears from the foregoing, that qualitatively, at least, a distinct correlation between the urinary output of ascorbic acid and the state of nutrition with respect to this substance had been well established. At the beginning of our work, we therefore undertook merely to apply the single daily dose technique of Harris, Ray and Ward (1933), as a means of studying and comparing

the state of ascorbic acid nutrition of rheumatic and non-rheumatic subjects. In preliminary studies, however, we found the initial response to single, daily, test doses, of subjects on adequate diets, to be so irregular that it seemed imperative to find out what constituted the *normal* response to high dose tests, by which all subjects could be graded.

At about that time, the work of Johnson and Zilva (1934) appeared. Their results, which we found similar to ours, were also obtained by feeding *varying* daily doses of orange juice. From their work and ours, it was evident that this procedure would yield no standard of normal excretion. Some of the irregular results in such experiments could be ascribed to the variation in ascorbic acid content of different lots of orange juice some of which we titrated after feeding.

In order to detect latent scurvy, of subclinical severity, it seemed necessary to develop a standardized technique of greater sensitivity, and capable of yielding more accurate quantitative results, than any procedure then available. Results so obtained, under average, normal conditions, could then be used as a measure of the state of nutrition with respect to ascorbic acid. We finally found it best to follow, in all subjects, the output response to repeated, large, single, daily doses of the pure substance. Because orange juice varies considerably in its ascorbic acid content, we used synthetic ascorbic acid in daily doses of 250 mgm. (The synthetic product "Redoxon" of Hoffmann-La Roche, and of Abbott, was found by titration to be the same as a sample of the natural crystalline material kindly sent us by Merck and Co.) Furthermore, since experiments with orange juice indicated that most individuals reached the peak of urinary excretion within seven days, the above dose was given daily for such a period. In this way, the effect of individual variations in immediate reaction to the first dose, perhaps out of proportion to the available body store of ascorbic acid, was eliminated. Furthermore, the additional factors of the level of urinary excretion at saturation, and the rate of increase in urinary output in the approach to that level, were brought within the scope of analysis. The following is a description of the procedure and the results when applied to rheumatic and non-rheumatic subjects.

## METHOD

*Diet.* Usually, immediately upon admission, the subject was given a diet comparatively low in ascorbic acid content, composed chiefly of cereals, eggs, cheese, and cooked lean meat. Milk, dried and canned fruit, and vegetables low in ascorbic acid, when fed, were thoroughly cooked. The amount of ascorbic acid in such a diet averaged no more than 12 mgm. daily, as calculated from the values for the various foodstuffs present which may still have retained some of the vitamin (Birch, Harris, and Ray (1933), Bessey and King (1933)). After 48 hours, the above diet was supplemented for the next seven days, by the addition of 250 mgm. of "Redoxon," taken after breakfast. The material was weighed out to  $\pm 1$  mgm. mixed with sugar in a spoon, and followed by a half glass of water. In order to obtain concentrated urine and a minimum number of samples, the fluid intake was restricted.

*Collection of urine specimens.* At the beginning of a test period, the urine voided at 8 a.m. was discarded. The last specimen for every 24 hour period was obtained at 8 a.m. It was not necessary to save all of the urine voided. Immediately after each voiding, a small sample was quickly transferred to a 30 cc. bottle which was stoppered tightly with a one-hole rubber stopper so that all air bubbles were driven out together with urine which overflowed through the aperture; this was then sealed by inserting a tightly fitting glass rod. The time of voiding and the volume were recorded, and the specimen was then put into the refrigerator. At the end of the 24 hour period, the collected samples were analyzed.

*Titration of urine specimens.* For analysis, one composite sample was prepared, representing a pooled, total 24 hour urinary output. Accordingly, from each sample bottle was pipetted a volume one-tenth or one-twentieth of the total amount of the patient's voiding represented by that sample. Thus, if five sample bottles were obtained from voidings of 180, 200, 300, 260, and 560 cc., samples of 9, 10, 15, 13, and 28 cc. were measured and mixed, giving a sample of 75 cc. of urine of the same composition as that which would have been obtained had all of the specimens been kept and mixed. In this way, the handling of large volumes of urine from several subjects, and the necessity of titrating each voided sample, were obviated. The proportionate samples were rapidly pipetted into a beaker containing mineral oil, quickly mixed by stirring, and immediately prepared for titration by the withdrawal of duplicate samples of 2 to 20 cc., depending upon the concentration of ascorbic acid present.

A volume of water to bring the total to about 48 cc., and 2.5 grams of trichloroacetic acid were added, and the mixture was quickly titrated against a dilute solution of 2:6 dichlorophenolindophenol added from a 3 cc. microburette. The end-point reached was a definite pink persisting for at least 30 seconds. This procedure of titrating definite volumes of urine by the addition of the indicator, was the reverse of that used by most workers, who have titrated definite volumes of indicator with the un-

known urine, to discharge the red color. The procedure employed by us, like that of Bessey and King (1933), seemed to yield a sharper end-point. Furthermore, when the titration was done in our way, the effect of trichloroacetic acid on the indicator was minimized. *Under these conditions, we found no difference in end-point of urine samples, whether trichloroacetic or glacial acetic acid was used for acidification.*

*Standardization.* The indicator solution was freshly prepared every 48 hours by repeated extraction of 0.15 gram of the solid material in warm water, and dilution to a volume of 200 cc. The solid, which dissolved almost completely, was the sodium salt of the indicator, prepared in this laboratory, according to the procedure of Gibbs, Cohen and Cannan (1925) as detailed by Bessey and King (1933). The solution was standardized, whenever used, against 5 cc. of a fresh solution of ascorbic acid, prepared by solution in water, and dilution to a total volume of 50 cc., of approximately 0.02 gram "Redoxon" accurately weighed. The indicator solution was then equivalent to from 0.35 to 0.4 mgm. ascorbic acid per cc., and urine samples required from 0.5 to 3.0 cc. of indicator per titration.<sup>2</sup>

*Calculation.* The standardization of the indicator was calculated from the equation

$$(1) \frac{\text{Mgm. ascorbic acid weighed for standard} \times 0.1}{\text{Cc. indicator to titrate 5 cc. of standard sample}} = f = \text{mgm. ascorbic acid equivalent per cc. indicator.}$$

The total daily output of ascorbic acid was calculated according to the equation

$$(2) \frac{\text{Cc. indicator to titrate urine}}{\text{Cc. urine sample}} \times f \times \text{cc. urine in 24 hours} = \text{mgm. ascorbic acid excreted in 24 hours.}$$

## EXPERIMENTAL

*Factors involved in the accuracy of the method*

*The specificity of the titration.* It is well recognized that the titration of biological fluids with 2:6 dichlorophenolindophenol may not yield results absolutely specific for ascorbic acid. Error arising from this source would produce higher values for ascorbic acid. However, Harris and Ray (1935) state that it is very unlikely that there are constituents present in normal urine in sufficient amount to interfere with the titration. We have noted that substances which may reduce the indicator, as does the added trichloroacetic

<sup>2</sup> The method of preparation as detailed, makes it impossible to avoid the presence of sodium chloride in the final material. Therefore, given weights of different batches of indicator may be expected to vary in their titration equivalent of ascorbic acid.

acid, do so at such a slow rate that their effect is rendered negligible by rapidity in titration. Tauber and Kleiner (1935) using a ferricyanide titration method, ascribe one-half of the total reducing power ( $\approx$  about 10 mgm. daily) of normal urine, to the presence of substances other than ascorbic acid. Ascorbic acid added to urine in their experiments was quantitatively recovered. The presence of substances other than ascorbic acid, capable of reducing the indicator in our titration, would make our results all high by a constant small amount, not affecting the comparative results for ingested ascorbic acid appearing in the urine.

*The preservation of ascorbic acid in urine.* Ascorbic acid in solution unprotected from air is very unstable, being rapidly oxidized. It seems that the stability of this substance in solution depends on the oxygen tension and the pH. Ascorbic acid in alkaline solutions is rapidly oxidized, but is relatively stable in the absence of oxygen. Acid solutions have been found to be but slightly affected by oxygen (Herbert, Hirst, Percival, Reynolds, and Smith (1933)).

In order to avoid or diminish loss by oxidation, other workers have found it necessary either to titrate all urine samples immediately after voiding, or to add acetic or sulfuric acid, as a preservative. In our studies, the former course proved to be impractical in the simultaneous study of several patients. Therefore, it was found necessary to determine the conditions under which small samples of every specimen passed, could be preserved for analysis on the following day. The results led us to adopt, as the most satisfactory from the standpoint of practicability coupled with accuracy, the method of collecting samples given in the preceding section.

The experiments in this connection indicated that in the preservation of ascorbic acid in urine, the three important factors were oxygen tension, temperature, and pH, the latter being of little consequence when the other two were properly controlled. Tables I, II, and III, representative of a larger number of similar results, serve to illustrate these points.

Table I indicates the decrease in ascorbic acid titration of urine samples left in contact with air for several hours. Acidification, either with

TABLE I  
*Change in ascorbic acid content of human urine exposed to air at room temperature, at different pH*

Sample number	Time	pH	Indicator titration	pH	Indicator titration
	hours		cc.		cc.
1	0		2.25		
	4		1.88		
2	0		1.16		
	4		0.88		
3	0		0.12		
	4		0.07		
4	0	5.4	1.74	4.6	1.73
	6½		0.89	(+ acetic)	0.94
5	0	7.2	0.74	4.6	0.75
	6		0.24	(+ acetic)	0.24
6	0	6.2	0.72	3.0	0.70
	23		0.34	(+ sulfuric)	0.34

TABLE II  
*Change in ascorbic acid content of human urine exposed to air at 10° and 25° C.*

Sample number	Time	pH	Indicator titration	
			At room temperature (25° C.)	At refrigerator temperature (10° C.)
	hours		cc.	cc.
1	0	6.7	0.86	
	6		0.55	0.79
	24		0.27	0.46
2	0	6.4	1.22	
	6		0.53	1.08
	24		0.36	0.99
3	0	4.2	0.67	
	23		0.43	0.58
4	0	7.6	0.67	
	23		0.52	0.56
5	0	6.1	0.72	
	24		0.34	0.52
6	0	3.0	0.70	
	24		0.34	0.42

acetic or sulfuric acid, did not prevent loss of titratable ascorbic acid.

It was found that at any pH from 3.0 to 7.6, loss of ascorbic acid on standing in contact with air could be considerably diminished by keeping the samples in a refrigerator. Table II contains the experimental evidence leading to this conclusion.

TABLE III

*Change in ascorbic acid content of human urine protected from air in mercury containers*

Sample number	Time	pH	Indicator titration		
			Sample as voided kept at 25°	Sample saturated with H <sub>2</sub> , kept at 25°	Sample as voided kept at 10°
	hours		cc.	cc.	cc.
1	0	6.4	1.20	1.20	
	6			1.14	
	24			1.11	
2	0	6.4			
	4			1.61	1.57
	6			1.61	1.57
	20			1.53	1.50
3	0	5.9	1.84		
	3		1.83	1.81	1.80
	5½		1.80	1.79	1.77
	22½		1.72	1.73	1.75
4	0	7.2	0.37		
	18		0.37		0.37
5	0	4.7	0.78		
	19		0.73		0.73
6	0	5.5	0.42		
	18		0.37		0.38
7	0	6.8	0.09		
	18		0.09		0.09
8	0	5.9	0.89		
	18		0.83		0.83

Finally, Table III indicates that the most important factor in preserving ascorbic acid in urine is undoubtedly the oxygen tension. In this series of experiments all samples were transferred immediately, after voiding under oil, to the sealed mercury containers of Austin *et al.* (1922) and kept under slight pressure of mercury. Air, therefore, had no access to the samples, and they were necessarily under their own oxygen tension (Table III, Columns 4 and 6). This tension, as Sendroy (1934) has observed, is quite low for most urines and sometimes diminishes on standing. Therefore, after exhaustion of the dissolved oxygen, either before or after voiding, barring a change to the reversible form, one would expect the ascorbic acid titer of urine to remain constant. As a matter of fact, we have found the titration value of several urines kept this way, even at room temperature, to be constant for two days, after a slight fall in the first 24 hours. Table III

also shows the relative unimportance of temperature under these conditions.

In Column 5 of the same Table, there are other examples of the preservation of ascorbic acid in urine, at practically zero oxygen tension. Immediately after voiding, these samples were saturated with hydrogen to the complete displacement of air. This method, first used, is probably the best, but is impractical for clinical work.

*The effect of amidopyrine and codeine dosage.* In the treatment of patients with rheumatic fever, the use of amidopyrine, and of codeine to a lesser degree, was frequently found advisable. Experiments were performed to control the effect of these drugs on the titration of ascorbic acid in excreted urine. The subjects used for this purpose had all been kept for some time on a daily dose of 250 mgm. of ascorbic acid, the feeding of which was continued during and after the daily intake of up to four times the usual doses of amidopyrine and codeine. Table IV, in a comparison of control periods immediately before and

TABLE IV

*The effect of amidopyrine and codeine dosage on the daily urinary output of ascorbic acid. Subjects in a state of saturation on a daily dose of 250 mgm. ascorbic acid.*

Case number	Daily dose		Average daily urinary output of ascorbic acid		
	Amount	Substance	Previous control 3 days	Dosage period 4 days	Post control 4 days
16-A....	0.9 gram	Amidopyrine	216	210	215
8-B....	0.9 gram	Amidopyrine	218	180	199
11-A....	1.8 gram	Amidopyrine	196	200	224
6-B....	1.8 gram	Amidopyrine	178	187	
11-B....	128 mgm.	Codeine	208	179	204
6-C....	128 mgm.	Codeine	203	164	168

after the dosage period of four days, indicates that amidopyrine had no effect on the results. The effect of codeine, if any, was to decrease the ascorbic acid output of the urine. In our studies, codeine was given in small doses, only when absolutely necessary. Error from this source would result in a slight tendency toward decreased ascorbic acid excretion for rheumatic fever cases in which the drug was used.

The foregoing observations of factors affecting the accuracy of the results, constitute the basis of the technique adopted for these studies. Loss

of ascorbic acid in urine samples was kept at a minimum by storage in sealed containers at low temperature. The error under these conditions was of the order demonstrated in Table III. Such slight losses probably balanced the positive error which may have been caused by the presence of small amounts of reducing substances other than ascorbic acid. Taking into consideration all sources of error, we estimate that our titrations approximated, within 10 to 15 per cent, the total ascorbic acid content of the urine.

### Results of urine studies

*Classification of subjects.* The complete data in connection with 33 studies made from January

to April, 1935, of the state of ascorbic acid nutrition of 28 individuals, are given in Table V. The subjects are separated into three groups.

In the first group are patients, afflicted at the time of the test, with active rheumatic fever, and its several accompanying conditions, such as pyrexia, polyarthritides, carditis, etc. The diet of some of these subjects had previously been supplemented with ascorbic acid feeding.

In Group II are patients who were not suffering from rheumatic fever at the time of the test, but who had previously had the disease. In this group are a few convalescent patients who had also been tested (Group I) while the disease was at its height, and some subjects (inactive rheu-

TABLE V  
Results of utilization tests of ascorbic acid on human subjects

1	2	3	4	5	6	7	8	9	10		11						12	13	14	15	16		
Case number	Sex	Age	Body weight	Condition	Fever	Gastro-intestinal symptoms	Medication	Previous (ascorbic acid) diet	Daily urinary ascorbic acid excretion on low ascorbic acid diet (less than 12 mgm. daily)						Total 7 days excretion of ascorbic acid	7 days intake minus excretion of ascorbic acid	C = Ascorbic acid unexcreted per kgm. body weight	√age	I = Utilization index = Column 14 X Column 15				
									2 days preliminary		7 days following with addition of 250 mgm. ascorbic acid by mouth, daily												
		years	kgm.		°F.				mgm.	mgm.						mgm.	mgm.	mgm.					
GROUP I—ACTIVE RHEUMATIC FEVER DURING TEST																							
1	M	4	10.5	Polyarthritides, pericarditis, carditis	100-104	Anorexia, repeated vomiting	Codeine	100 mgm. daily, 1 month	Good	65	56	113	200	38	56	88	58	82	635	1109	61.5	2.00	123.0
2	M	7	21.5	Polyarthritides, pericarditis, pleurisy, pneumonia(?)	100-103.4	Anorexia, nausea, vomiting	Codeine	100 mgm. daily, 2½ months	Very good	16	16	24	29	18	23	37	45	48	224	1810	74.0	2.64	107.7
3	F	7	28.8	Polyarthritides, myocarditis	99-104.6	Anorexia	Codeine	Poor		24	17	14	33	52	78	154	219	201	754	1090	37.5	2.64	99.0
4	F	7	22.0	Polyarthritides, myocarditis	99-104.5	Anorexia, abdominal pain	Codeine	100 mgm. daily, 2½ months	Very good	35	38	60	132	185	180	144	163	110	973	861	30.1	2.64	103.2
5	F	9	41.4	Polyarthritides, pleurisy, myocarditis	99-104.5	None	None	Good		43	24	32	131	180	201	182	218	227	1169	665	16.1	3.00	48.3
6	M	10	36.4	Slight polyarthritides, carditis	99-100.5	None	None	Good		25	26	55	113	165	194	212	205	180	1123	711	19.5	3.16	61.6
7	M	11	57.7	Polyarthritides, myocarditis	100-105	Anorexia	Codeine	Very good		43	41	57	96	95	104	93	96	102	643	1191	20.7	3.32	68.6
8	M	15	40.8	Polyarthritides, myocarditis, jaundice	100-104.5	Anorexia	Codeine	Poor		103	107	116	109	163	145	125	125	140	931	903	18.1	3.87	70.2
9	M	15	44.0	Myocarditis, congestive failure, pneumonia(?)	100.2-105	Anorexia, vomiting	Codeine, amidopyrine	100 mgm. daily, 3 months	Poor	122	83	61	87	69	94	148	212	214	834	950	21.6	3.87	83.6
10	M	16	45.0	Polyarthritides, myocarditis	99-103.5	Anorexia, vomiting	Codeine	Good		33	22	53	48	72	115	160	220	193	859	975	21.7	4.00	80.8
11	M	17	54.2	Myocarditis, partial heart block	99-100	None	None	Good		25	26	33	75	132	175	186	178	163	947	837	15.4	4.12	61.4
12	F	19	59.0	Polyarthritides, carditis(?)	99-100.5	Anorexia	None	Very poor		20	22	21	25	27	21	32	33	73	238	1595	27.1	4.35	114.0
13	F	20	44.8	Myocarditis, low grade polyarthritides	99-100.5	Anorexia, vomiting	Codeine	250 mgm. daily, 4 months		49	18	61	138	102	144	153	71	99	795	1039	23.2	4.47	103.8

TABLE V—Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Case number	Sex	Age	Body weight	Condition	Fever	Gastro-intestinal symptoms	Medication	Previous (ascorbic acid) diet	Daily urinary ascorbic acid excretion on low ascorbic acid diet (less than 12 mgm. daily)		Total 7 days excretion of ascorbic acid	7 days intake minus excretion of ascorbic acid	C = Ascorbic acid excreted per kgm. body weight	√age	I = Uric acid index = Column 14 X Column 15
		years	kgm.		°F.				2 days preliminary	7 days following with addition of 250 mgm. ascorbic acid by mouth, daily	mgm.	mgm.	mgm.	mgm.	mgm.

## GROUP II—CONVALESCENT AND INACTIVE RHEUMATIC FEVER

3-A	F	7	28.8	Convalescent R.F.*	99.4-100	None	None	250 mgm. daily, 7 weeks	29	21	144	168	160	191	181	209	211	1264	570	19.8	2.64	52.3
14	M	8	25.5	No R.F. symptoms, varicella	99-100.5	None	None	250 mgm. daily, 7 weeks	29	20	137	145	156	173	168	176	157	1133	701	27.5	2.83	77.6
15	F	9½	29.0	Acute bronchitis	None	None	Digitalis 0.1 gram daily	100 mgm. daily, 3 months	17	22	152	170	207	174	198	181	190	1272	562	19.4	3.08	59.8
6-A	M	10	35.4	Convalescent R.F.	99.8-100	None	None	250 mgm. daily, 3 months	50	33	280	189	235	212	214	237	155	1535	279	7.66	3.16	24.2
16	F	10	30.1	Convalescent R.F.	99.5-100	None	None	250 mgm. daily, 5 weeks	29	23	169	171	185	195	181	212	191	1305	528	17.5	3.16	53.4
17	M	13	38.1	Convalescent pharyngitis	None	None	None	100 mgm. daily, 1 month	35	27	62	142	167	227	187	176	158	1119	715	18.8	3.60	67.6
8-A	M	15	53.4	Convalescent R.F. (No jaundice)	None	None	None	250 mgm. daily, 1 month	55	42	86	140	182	158	191	195	208	1156	678	12.7	3.87	49.1
18	M	17	56.0	Carditis?	99-101	None	None	100 mgm. daily, 6 weeks	38	38	47	83	127	147	195	177	195	974	860	15.4	4.12	63.2
19	M	17	60.4	Catarthal jaundice	None	Anorexia	None	100 mgm. daily, 11 days	102	118	116	144	145	114	132	98	97	845	989	15.4	4.12	67.4
20	F	19	37.0	Perniciious anemia	None	Anorexia, nausea, vomiting	None	Poor	47	41	82	81	119	139	158	114	130	823	1011	27.3	4.35	112.0
20-A	F	19	38.5	Convalescent perniciious anemia	None	Vomited only once	None	Fair	53	56	94	125	156	154	173	165	157	1034	800	20.8	4.35	90.7
2-A	F	19	59.0	No R.F.	None	None	None	250 mgm. daily, 5 weeks	43	29	97	130	110	137	164	140	176	953	831	14.9	4.36	65.1
21	M	21	63.0	Recovered R.F. chronic valvular disease	None	None	None	100 mgm. daily, 1 month	27	50	53	85	124	184	192	188	200	1029	805	12.8	4.27	58.8

\* R.F. = Rheumatic fever.

† Case 3-A = Case 3, after 50 days; Case 6-A = Case 6, after 3 months; Case 8-A = Case 8, after 1 month; Case 20-A = Case 20, after 1 month; Case 12-A = Case 12, after 5 weeks.

## GROUP III. NORMALS AND NEGATIVE RHEUMATIC FEVER HISTORIES

22	F	8½	15.6	Vaginitis	99-100	None	None	Very poor	98	88	12	14	25	139	189	113	120	614	1279	78.2	1.94	151.6
23	M	8½	25.7	Convalescent mumps	None	None	None	Good	16	15	175	140	170	217	209	162	155	1253	869	22.1	2.92	64.7
24	M	8½	26.7	Convalescent varicella	None	None	None	Good	35	29	95	185	102	166	182	175	167	1083	751	23.1	2.96	83.2
25	M	17	46.1	Convalescent lobar pneumonia	None	None	None	Poor	31	24	29	35	40	75	95	145	182	622	1222	26.7	4.12	110.9
26	M	28	52.9	Convalescent lobar pneumonia	None	None	None	Good	41	21	89	172	190	187	170	124	178	1149	685	12.9	5.29	64.2
27	M	28	81.8	Normal		Indigestion 6th day	None	Good	37	49	75	52	85	125	151	120	131	740	1264	12.4	5.29	71.9
28	M	34	65.8	Normal			None	Good	37	37	63	139	155	175	177	185	173	1156	685	13.4	5.84	61.7



matic fever) who had been followed in our outpatient clinic, before being admitted to the wards for these studies. All of the members of this group, with one exception (Cases 20 and 20-A) had been taking ascorbic acid for varying lengths of time, prior to the test. This, and their freedom from their previous infection, made them desirable as control subjects.

Group III comprises two healthy individuals and several patients in a more or less advanced state of convalescence from diseases other than rheumatic fever. None of this second control group had ever suffered from rheumatic fever.

*The selection of controls for the relative standard of normality of ascorbic acid nutrition.* An absolute standard of the state of ascorbic acid nutrition in human beings, based on studies of urine excretion, would be the result obtained by a high dose study of individuals previously kept for some time on a controlled diet containing a definite amount of ascorbic diet very nearly the minimum adequate for the maintenance of good health. Results which varied markedly from the average of a large number of such studies could then be interpreted as an indication of a condition of ascorbic acid deficiency or excess. Since the establishment of such a standard offers obvious difficulties we decided to base our standard of ascorbic acid nutrition on the results obtained in a study of individuals previously on an average diet, the subjects being free from digestive or metabolic disturbances during the high dosage test. Since we were interested not in absolute so much as relative results, the use of control subjects taken from Groups II and III was justified, even though the intake of ascorbic acid of the former group, if not of the latter also, probably exceeded the actual minimum adequate requirement.

To arrive at an average normal standard, it seemed logical and necessary to exclude cases known to have been for a long period on a diet extraordinarily high or low in ascorbic acid. Cases Number 20, 22, and 25 were therefore omitted because of a previous dietary deficiency, and Cases 3-A, 6-A, 16, 8-A, and 12-A were also dropped here, because the subjects had taken over 250 mgm. ascorbic acid daily for several weeks. In our judgment, the other members of Group II had received wholly adequate, although

not excessive, quantities of ascorbic acid, in that their habitual diets had been supplemented with 100 mgm. of ascorbic acid daily for periods of eleven to sixty days.

*The calculation of a relative or reference standard of ascorbic acid nutrition.* An inspection of Column 10, Table V, for all cases, shows that a study of the urinary excretion of ascorbic acid for two days on a low ascorbic acid diet is an unreliable and at best an insensitive indicator of the previous diet of the individual. Apparently, no reasonable quantitative standard of normal excretion can be derived from these results on any basis. In this, we are in agreement with previous workers.

If the procedure of Harris, Ray and Ward (1933), and of Harris and Ray (1935) be followed, and an attempt be made to determine the ascorbic acid saturation of the subjects by the response to one test dose (1st excretion value in Column 11, Table V) a qualitative index is obtained, unsuitable for quantitative comparisons. The latter authors have themselves suggested that it might be of more value to study the excretion further. This, we have done. For reasons previously discussed, it seemed that the total of seven days excretion on a daily high test dose of 250 mgm. would be more informative.

The total quantities excreted, however (Column 12, Table V), in themselves, reveal little that can be used in a systematic classification of these results. It seemed possible that the difference between the amounts of intake and of excretion during the test period might be of considerably greater importance and more distinctly characteristic of the body nutrition, than the excess (not used by or lost in the body) appearing in the urine (Column 13, Table V). Furthermore, since ascorbic acid, despite its relatively small total amount, is very widely, although irregularly, distributed throughout the body (Bessey and King (1933), Phillips and Stare (1934)), and seems necessary for the normal functioning of body processes (Bessey and King (1933), Szent-Györgyi (1934)), it appeared logical that this utilization, real or apparent, be calculated on a body weight basis. When this was done (Column 14, Table V), these utilization coefficient values for our eleven control cases apparently varied with the age of the subject.

Further examination indicated that there is an inverse relationship between the square root of the age of the subject and the *utilization coefficient*. Thus the results follow the equation

$$(3) \quad C \times \sqrt{\text{age}} = I,$$

where  $C$  is the *utilization coefficient*, or (mgm. ascorbic acid intake — mgm. ascorbic acid urinary output)/(kgm. body weight). Age is given in years, and  $I$  is the *utilization index*. Thus, the value of  $I$  for any subject, as compared with the average value of  $I$  for *control* subjects, is an indication of that individual's state of ascorbic acid nutrition. Values of  $I$  higher than the average indicate a *relative* ascorbic acid deficiency or subnutrition, and lower ones, a *relative* ascorbic acid excess.

A calculation of values of  $I$  (Column 16) for control subjects *previously* with an *approximately normal* ascorbic acid intake (with the omission of

Case 20-A, which definitely seems outside of the normal limit of variation, and which will be discussed later), gives an average of 65.8 for the six such subjects of Group II, and of 69.5 for the five similar subjects of Group III. Thus it seems, from these results, that the state of ascorbic acid nutrition of the members of Group III on average, good, mixed diets, approximated the condition of members of Group II who had been taking over 100 mgm. of pure ascorbic acid daily, by mouth.

The control points, plotted in Figure 1, follow the curve of average ascorbic acid utilization in our method, represented by the equation

$$(4) \quad C \times \sqrt{\text{age}} = 67.5$$

with an average deviation of  $\pm 5.5$ . The two outer curves define the limits of "normal" results, at least, between the ages of 7 and 35 years. Areas D and E therefore correspond to conditions

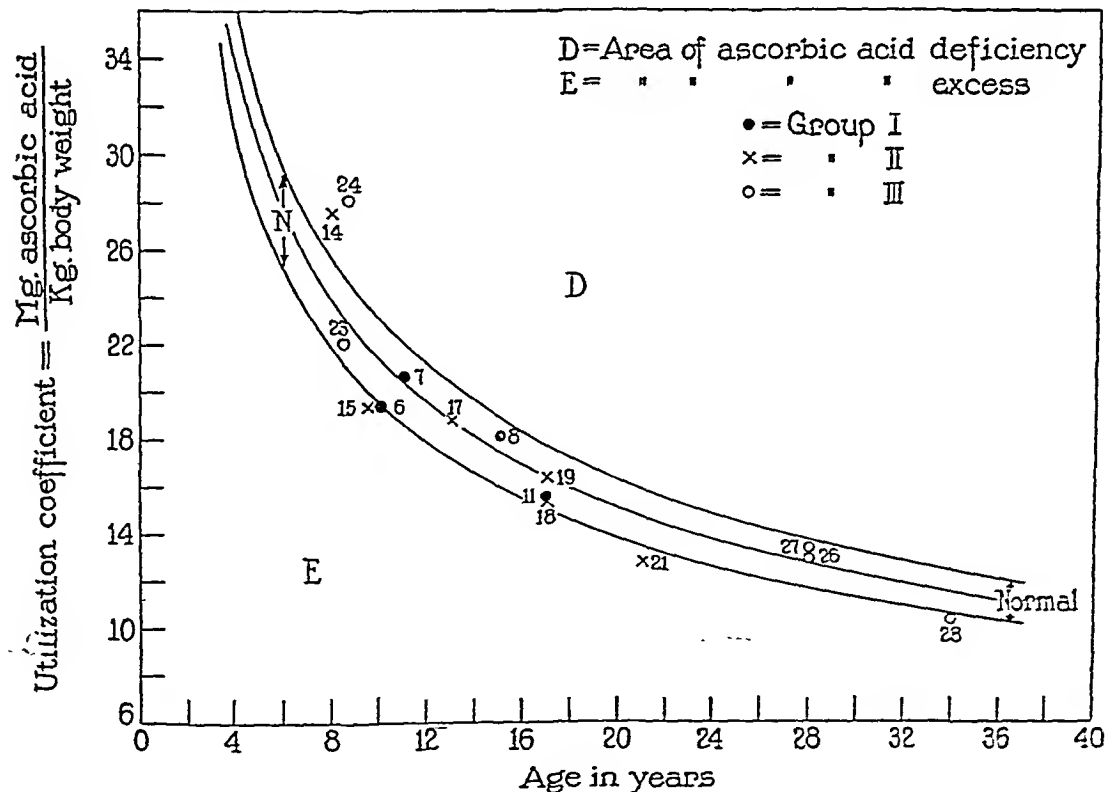


FIG. 1. DATA FROM TABLE V. THE VARIATION IN THE UTILIZATION OF ASCORBIC ACID FOR SUBJECTS OF GROUPS II AND III, ON AVERAGE GOOD DIETS WITHOUT DIGESTIVE DISTURBANCES.

Four cases of Group I showing normal results are also included. Case numbers are marked for each point.

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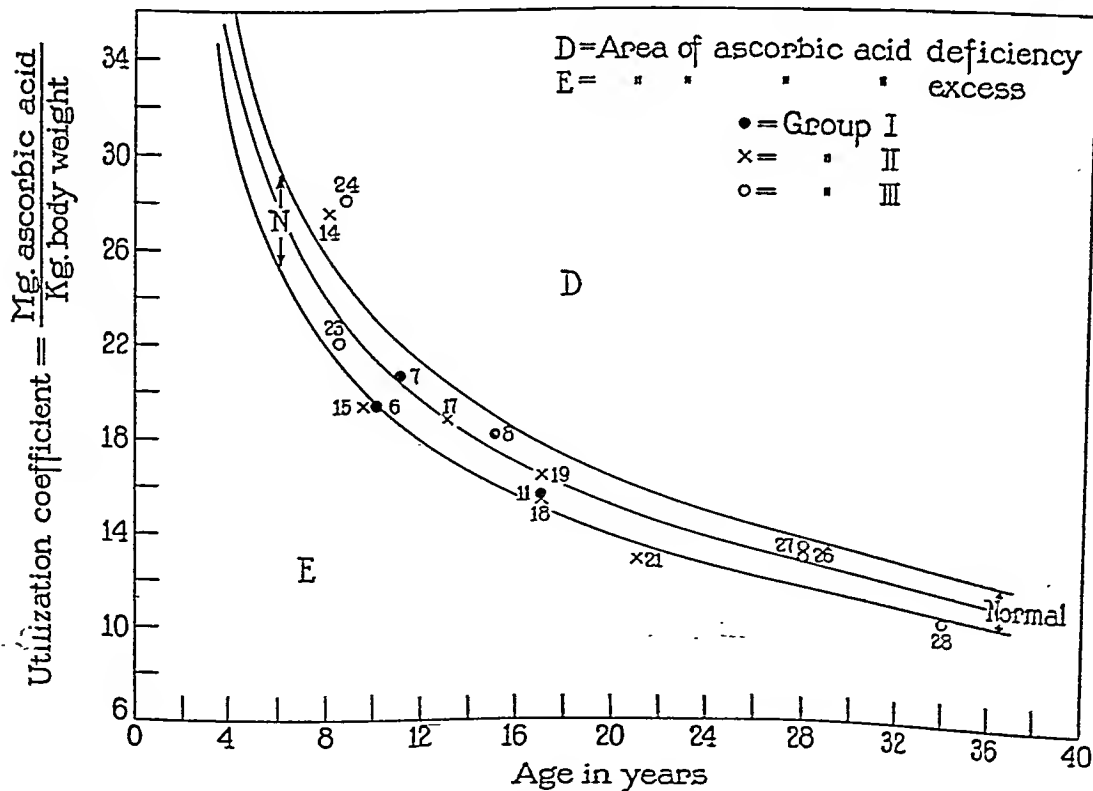


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Four cases of Group I showing normal results are also included. Case numbers are marked for each point.

of *relative* ascorbic acid deficiency and excess, respectively. Naturally, on the basis of these results, it cannot be claimed that the relationship of age to ascorbic acid utilization given by Equation 4, is the true mathematical expression for the physiological processes involved in the metabolism of ascorbic acid. The equation is a convenient, empirical expression which happens to fit the data well.

Since we had no opportunity of studying normal children below the age of seven, the course of the curve below that age is very doubtful. Indeed, the results of Hess and Benjamin (1934)

indicate that there may be a marked difference in the ascorbic acid metabolism and requirement of infants, in comparison with adults.

*Analysis of the results of ascorbic acid utilization of subjects with rheumatic fever.* Of the 13 patients with rheumatic fever studied at the height of their infection, 8 were found relatively deficient (Cases 1, 2, 3, 4, 9, 10, 12, 13), 4 were normal (Cases 6, 7, 8, and 11), and one was above normal (Case 5), in ascorbic acid nutrition, as judged *solely* by the values of the utilization index (Table V, Column 16), or the position of the points plotted in Figure 2.

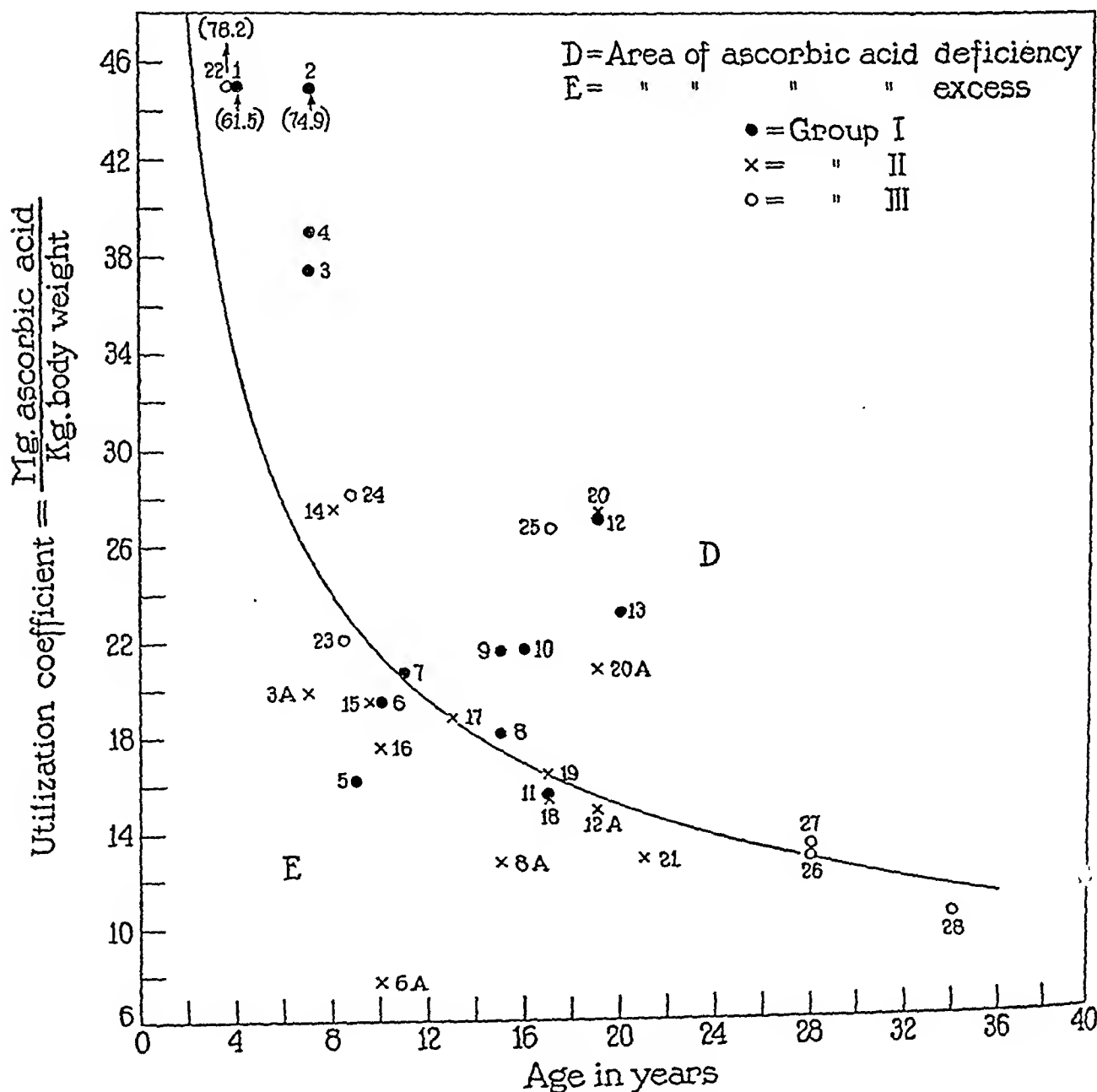


FIG. 2. DATA FROM TABLE V. THE RESULT OF ASCORBIC ACID UTILIZATION TESTS FOR ALL SUBJECTS STUDIED.

In our discussion of previous work, and the evaluation of a normal reference standard in the preceding section, it has been emphasized that the urinary excretion test of ascorbic acid is largely affected by two factors, the *first* of which is the *relative state of saturation* of the tissues of the individual at the beginning of the test. This, in turn, is mainly dependent on the ascorbic acid content of the previous diet, the proportion of the ingested material which was absorbed, and the proportion of the absorbed material which was stored. There is an additional possible factor, it seems, which would affect the results, but concerning which we have no definite indication, since the result would be the same as that indicating a low state of saturation caused by a poor supply or faulty absorption, namely, a condition in which ascorbic acid may be used up or metabolized in excess of normal amounts. It seems quite possible, from these considerations, that a condition of deficiency, as indicated by a relatively low state of saturation, may arise from causes other than a quantitative deficiency in the amount of ascorbic acid taken in the diet.

The *second* factor, it must be recalled, affecting the urinary excretion of ascorbic acid, is the *intake* in the diet of the subject *during the course of the test*, when the urine is being analyzed. In the preceding section it has been shown that if this second factor can be kept *constant*, by the ingestion and absorption of fixed high doses of ascorbic acid, then the results may be taken as a measure of the first factor, namely, the state of saturation. If this second factor is not kept constant, as in the feeding of orange juice of varying ascorbic acid content, or in the case of subjects incapable of assimilating the substance, the results will be invalid as an indication of the state of saturation.

In the search for a possible connection between the occurrence of rheumatic fever and any condition of ascorbic acid deficiency resulting in a low state of saturation relative to normals, the interpretation of the results requires that possible variations in the second factor be taken into consideration. Of the subnormal rheumatic cases, only *three* (Cases 3, 10 and 12) had been on a very poor ascorbic acid-containing diet, such as would be expected to lead to a condition of deficiency. Of the other subnormals, *previously on average to very good diets* with respect to ascorbic acid con-

tent, four exhibited conditions of vomiting, anorexia, and general gastric disturbance. There was no vomiting in Case 4, but anorexia.

We have no data as to how much, if any, ascorbic acid was lost during vomiting. But two conclusions are inevitable from those considerations, namely, that a gastro-intestinal disturbance, if it occurs during the feeding period of the test (second factor), invalidates the results; if it occurs (and reoccurs) for some period immediately preceding the test (first factor) it may well lead to an actual condition of relatively low ascorbic acid saturation, like that brought about by a dietary deficiency.

With five results invalidated on account of vomiting (but one of these had previously been on a poor ascorbic acid diet), we find (Table VI)

TABLE VI

*The effect of previous diet and of digestive and nutritional disturbances on the result of the ascorbic acid feeding test*

1	2	3	4	5	6	7
Group number	Case number	Previous diet with respect to ascorbic acid	Nutritional disturbance during the test	Result of nutrition test*		
				Found	Expected on basis of diet	Expected on basis of diet and condition during test
I	1	Good	Much vomiting, anorexia	D	N	D
	2	Good	Much vomiting, anorexia	D	D	D
	3	Poor	Anorexia	D	D	D
	4	Good	Malnutrition, anorexia	D	D	D
	5	Very good	None	E	E	E
	6	Good	None	N	E	N
	7	Very good	None	N	E	E
	8	Poor	None	N	D	D
	9	Good	Vomiting, anorexia	D	N	N
	10	Poor	Vomiting, anorexia	D	D	D
	11	Good	None	N	N	N
	12	Poor	Malnutrition, anorexia	D	D	D
	13	Very good	Vomiting, anorexia	D	E	D
II	3-A	Very good	None	E	E	E
	14	Good	None	N	N	N
	15	Good	None	N	N	N
	6-A	Very good	None	E	E	E
	17	Very good	None	E	E	E
	18	Good	None	N	N	N
	8-A	Very good	None	E	E	E
	19	Good	None	N	N	N
	20	Good	Anorexia	N	N	N
	20-A	Very poor	Vomiting, anorexia	D	D	D
	12-A	Good	Vomiting, anorexia	D	N	D
III	21	Very good	None	N	E	E
	22	Good	None	N	N	N
	23	Good	None	N	N	N
	24	Good	Anorexia	D	N	D
	25	Poor	Malnutrition, anorexia	D	D	D
	26	Good	None	N	N	N
	27	Good	None	N	N	N
	28	Good	None	N	N	N

\* N = Normal.  
D = Ascorbic acid deficiency.  
E = Ascorbic acid excess.  
X = Agreement with result found.

among the remaining eight cases of rheumatic fever, but *two* cases of deficiency caused by previous diet (Cases 3 and 12) and one (Case 4), with a condition of low saturation caused by some other factor, associated with a nutritional disturbance, before or during the test.

Table VI, Column 6, shows that in 23 out of 33 cases, the result of our test was in agreement with what would have been expected on the basis of our knowledge concerning the subjects' previous diet. When the probable depressant effect of digestive and nutritional disturbances on the ascorbic acid intake, and storage, is also taken into consideration (Column 7) the agreement is still closer, being 29 out of 33. The exceptions to this agreement are found in Cases 7, 8, 19, and 12-A, which will be mentioned again in the following sections.

*The effect of icterus on the results of urinary excretion of ascorbic acid.* It is interesting, and probably not without considerable significance, that Cases 8 and 19 were exact parallels in many respects. These two male patients, of about the same age and weight, were both jaundiced, showed bile in urine and serum, and had pyrexia. On the other hand, Case 8 had active rheumatic fever, and had previously been on a very poor diet, while Case 19 had no sign of the disease and had previously taken at least 100 mgm. ascorbic acid for 11 days. Yet, insofar as the ascorbic acid utilization test is concerned, both showed a parallel behavior quite different from those of any other class of patients.

The initial excretion for the first two days of substance reducing the titration indicator, without any intake of ascorbic acid, was high in both cases. Furthermore, the feeding of 250 grams for the next 7 days resulted in but a slight rise in the titration values in each case. A calculation of the utilization index showed values for both which were higher than those which would have been expected on the basis of diet alone. Furthermore, when Case 8 convalesced later (Case 8-A) and showed *no* icterus, the excretion picture was like that of the other patients of Group II, and the utilization index was entirely in line with the results of other subjects after a high feeding diet. In cases of icterus, the abnormal titration of the urine may be caused either by the increased excretion of reducing substances other than ascorbic

acid, or by a real disturbance in the ascorbic acid excretion or utilization processes. This point bears further investigation.

*The effect and significance of the results of intravenous injections of ascorbic acid following oral feeding.* In a state of *saturation to any fixed dose* of ascorbic acid, an individual will approximate a condition in which there is only a relatively small, fairly constant difference between intake and urinary excretion, representing the amount stored, metabolized, or lost in the tissues. Such a condition was shown by but few of our subjects. Johnson and Zilva (1934) used as a criterion of saturation, the *constancy* of the level in urinary excretion finally attained on a constant daily intake. However, this did not seem satisfactory enough, since we were interested in obtaining some idea as to what happened to the material *unexcreted* in the urine when there was apparently a condition of saturation, requiring little further use or storage of the substance. Johnson and Zilva (1934) have already shown that this apparent loss could not be recovered or identified in the urine as dehydroascorbic acid.

In order to make certain of the intake, and to eliminate the factor of gastro-intestinal absorption, we tested some of our subjects by giving intravenous injections of 250 mgm. ascorbic acid for 2 days following the usual feeding period of 7 days. To prevent oxidation of the material during handling, the solutions used for injection were prepared and sterilized immediately before use, as follows: The ascorbic acid was accurately weighed out (500 mgm.), and placed in a 10 cc. calibrated Pyrex flask. A volume of 5 cc. of 1.8 per cent NaCl was added, *without stirring*, the neck was stoppered with cotton, and the flask was immersed to the volume mark in a Crisco oil bath at 120° C. After 10 minutes, during which time the powder dissolved completely, the flask was cooled and the solution was made up to volume with water, under sterile conditions. The solution was mixed and taken up in a syringe from which the subject received 5 cc. The entire procedure, from sterilization to injection, required no more than 20 minutes. Contrary to the assertion of Wright and Lilienfeld (1936) that "we are dealing with a substance which is destroyed by heat and hence cannot be completely sterilized," immediate titration of solutions prepared and used in this way

showed a maximum loss of only 2 per cent of the material weighed.

Table VII shows that every subject so tested, excreted more ascorbic acid in the urine while re-

TABLE VII

*Results of intravenous injections of ascorbic acid following oral administration. Dose in all cases, 250 mgm., present in diet, 12 mgm.*

Group number	Case number	Daily urinary excretion of ascorbic acid		Added increase in injection over previous period	Conclusion
		Period previous to injection	Injection period		
		mgm.	mgm.	per cent	
I	2	37, 45, 48	154	253	Loss in feeding
	4	60, 132, 153, 180, 144, 163, 110	255, 207	45	Poor absorption
	6	203, 164, 173	213, 274, 156, 243	23	Oral saturation
	7	104, 93, 96, 102	150, 224	104	Poor absorption
	9	212, 214	195, 293	16	Oral saturation
	10	220, 193	221, 251	14	Oral saturation
	11	225, 212, 188	234, 198, 195, 283	9	Oral saturation
	13	84, 138, 102, 144, 153, 71, 99	186, 211	68	Loss in feeding
	6-A	236, 212, 214, 237, 186	219, 223	4	Oral saturation
	16	211, 191	220, 289	25	Oral saturation
II	8-A	191, 193, 203	245, 219	17	Oral saturation
	18	193, 177, 195	233, 213	18	Oral saturation
	20-A	173, 165, 167	235, 229	39	Loss in feeding
	12-A	164, 140, 176	181, 221	23	Oral saturation?
	21	181, 192, 188, 200	210, 202	8	Oral saturation?
	Average of 10 cases saturated orally.....			16	

ceiving the ascorbic acid intravenously, than during a previous feeding period. This is the case even for subjects who were presumably in a state of saturation at the time of the injections, i.e., they had reached a maximum in urinary output while being fed. These results indicate that there is some loss of the substance in the alimentary tract under all conditions of ascorbic acid nutrition.

As judged by the excretion values for the two periods, of the 15 cases, 10 were near or at saturation by oral feeding, at the time of injection. For these cases the average increase in the daily urinary output of ascorbic acid after injections, was 16 per cent, in no case exceeding 25 per cent. On the other hand, the comparatively large increases in the others, seem to indicate either a previous greater loss of the substance in the alimentary tract, or a greater storage or loss in the tissues.

That it was a matter of absorption (associated with anorexia, etc.) is clearly shown by reference to Table V, Column 7, and to the sudden high level (in all cases but that of Case 2) reached on

injection. Had the lowered excretion during feeding been the result of storage or destruction in the tissues, injection would not have caused such an abrupt cessation of the process. As a matter of fact, Cases 2, 13, and 20-A undoubtedly lost much of what was fed by vomiting. Case 2 apparently received so little during the feeding period, that the body stores were depleted and the injected material was retained to a much greater degree (Column 4), as compared with the excretion in the injection period of the other subjects of Table VII. In Cases 4 and 7 the inference is clear that for some reason there was a decreased absorption of ascorbic acid during the test. Considering the diet previously given, Case 12-A probably also had a subnormal absorption.

In this connection, it is interesting to note that Hou (1935) has found that the degree of protection given to guinea pigs on subminimal protective doses of ascorbic acid, was twice as much when the material was subcutaneously injected, as when given orally.

From these experiments, it seems that the feeding procedure for the determination of the state of ascorbic acid nutrition may sometimes indicate an apparent deficiency where there really is none. For this reason, it would be desirable to have for diagnostic purposes, a procedure which would eliminate the complicating factor of disturbances along the alimentary tract occurring *during the test*. The result of such a test would then be entirely a function of the state of saturation of the tissues. The factors of previous diet and of absorption of the ascorbic acid in that diet, contributing toward the result, could then be evaluated by further feeding tests, in order to determine whether oral or intravenous therapy was necessary to make up the tissue deficiency. Work on such a procedure will be undertaken in this laboratory.

#### DISCUSSION

Applied to the subjects with rheumatic fever, the excretion tests, relative to the controls, indicated to some degree, an apparent ascorbic acid deficiency in 8 out of 13 cases. Of these, the result in only 2 cases (Cases 3 and 12) could be ascribed solely to poor diet. In the other 6 cases, vomiting occurred, or else there was an incomplete absorption from or destruction of ascorbic acid



in the alimentary canal during the test (Case 4). Apparently, even in control cases, when there was a nutritional disturbance or anorexia during the test, the ascorbic acid, regardless of the previous diet, was not well assimilated, and was destroyed to a greater extent than usual.

Through digestive disturbances, patients with rheumatic fever evidently may develop a real hypovitaminosis on an ordinarily adequate diet. One would expect in such patients, that the tissues might be depleted not only of ascorbic acid but of other vitamins and essential food constituents of which there may not be large reserves in the body. However, even if we assume, contrary to the evidence of our experiments, that it is simply ascorbic acid deficiency that is associated with every case of rheumatic fever, it seems much more reasonable to regard the train of events, including digestive disturbances, leading to such depletion of the tissues, as caused by an infectious process, rather than to think of the ascorbic acid deficiency as initiating the infection. It seems certain that the factor of infection is present in all cases of rheumatic fever, whereas the signs of ascorbic acid subnutrition, if present, are probably incidental. Furthermore, it should be noted again that such signs of deficiency as have been found are only *relative* and not absolute. It has already been pointed out that our average results set up too high a standard of normality so that degrees of ascorbic acid deficiency, relative to the control cases previously on diets of about 100 mgm. ascorbic acid daily, would be exaggerated in the direction of ratings too low for ascorbic acid nutrition. When all of these factors are taken into consideration, it is difficult to accept subclinical scurvy as an etiological agent in rheumatic fever.<sup>4</sup>

The authors are indebted to Dr. Homer F. Swift for his advice and criticism during the course of the work, and in the preparation of this and the following paper.

<sup>4</sup> As this paper goes to press, we note the recent communication of Perry (1935), who used the single dose method of Harris, Day, and Ward (1933), studying the 12 hour urinary output after oral administration of 500 mgm. of ascorbic acid. He concludes from a study of 5 active, and 6 quiescent cases of rheumatic heart disease, that "vitamin C deficiency is not an important factor in the causation of acute rheumatism."

## SUMMARY

The urinary excretion test for the adequacy of ascorbic acid nutrition has been improved and placed upon a quantitative basis by a chemical and clinical study of the various factors affecting the final results.

Applied to a comparison of patients with rheumatic fever and control subjects, the results of this method do not support the concept that a condition of ascorbic acid deficiency is a predisposing factor in the causation of this disease.

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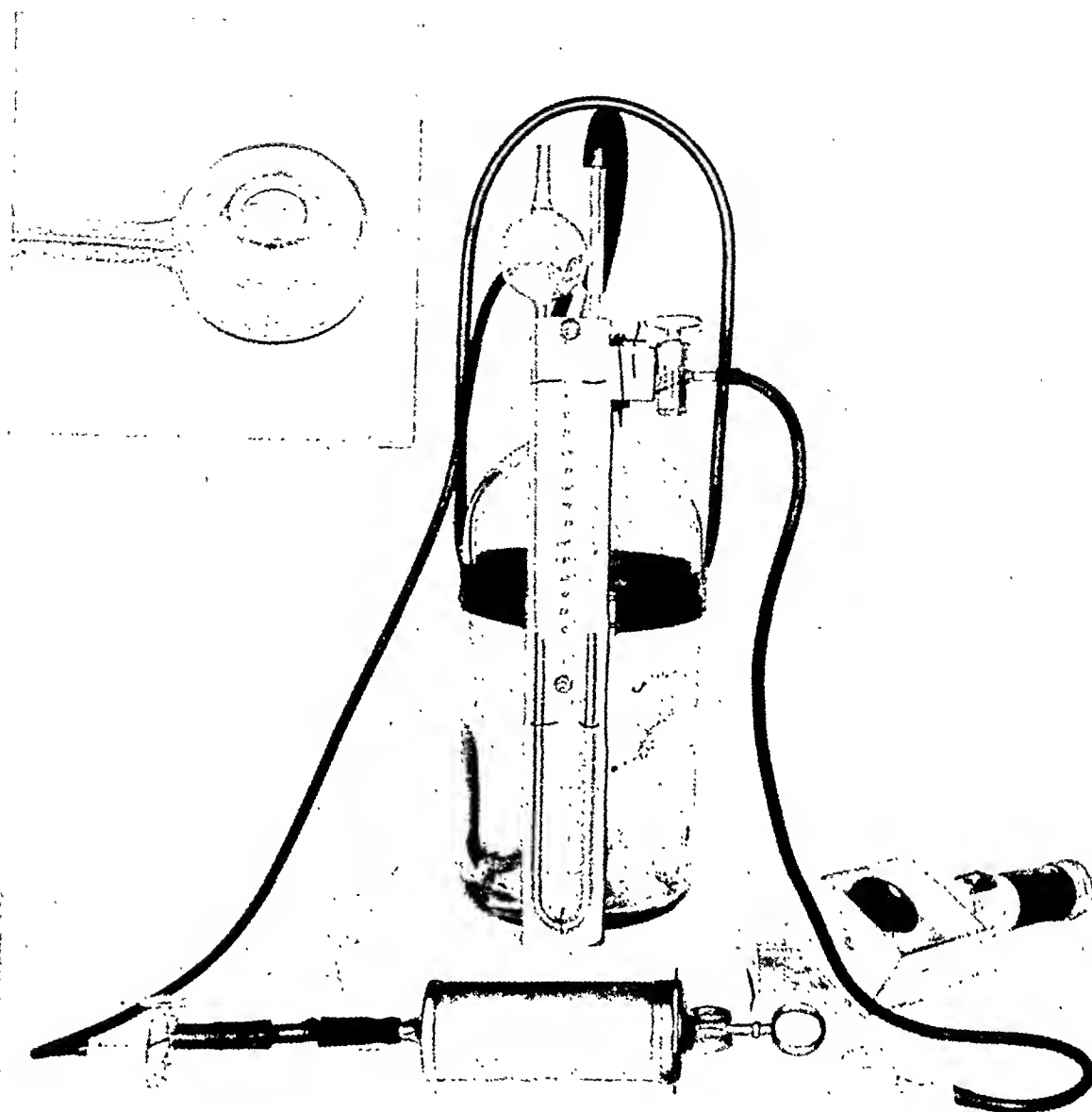


FIG. 1. APPARATUS USED IN THE DETERMINATION OF CAPILLARY PERMEABILITY.

Note detail of suction cup in insert: Diameter of suction area—1 cm. Width of flange resting upon cutaneous surface—1 cm.

at intervals of 10 mm. Hg were investigated; and the reading recorded in each instance was the lowest negative pressure at which discrete capillary hemorrhages appeared.

#### *Incidence of abnormal capillary fragility*

Great individual variations in the amount of negative pressure required to produce capillary hemorrhage were evident. The fact that many racial groups were represented among these patients may, in part, account for this, for it was noted that readings were often high in subjects with heavily pigmented skins. For this reason no absolute standard could be used, and in each subject changes relative to the preliminary deter-

mination in January were taken into account. Readings in patients with intercurrent febrile disease other than rheumatic fever were discarded. As shown in Figure 2, the degree of negative pressure required to produce capillary hemorrhage in the control Group B decreased on the average, especially from January to April, indicating that there was a relative increase in capillary permeability during the late winter and early spring. In contrast there often was noted in the other group, a slight decrease in capillary fragility which usually appeared following the taking of ascorbic acid; and this decrease was maintained during the remainder of the period of observation (see Figure 3). These findings indicate that

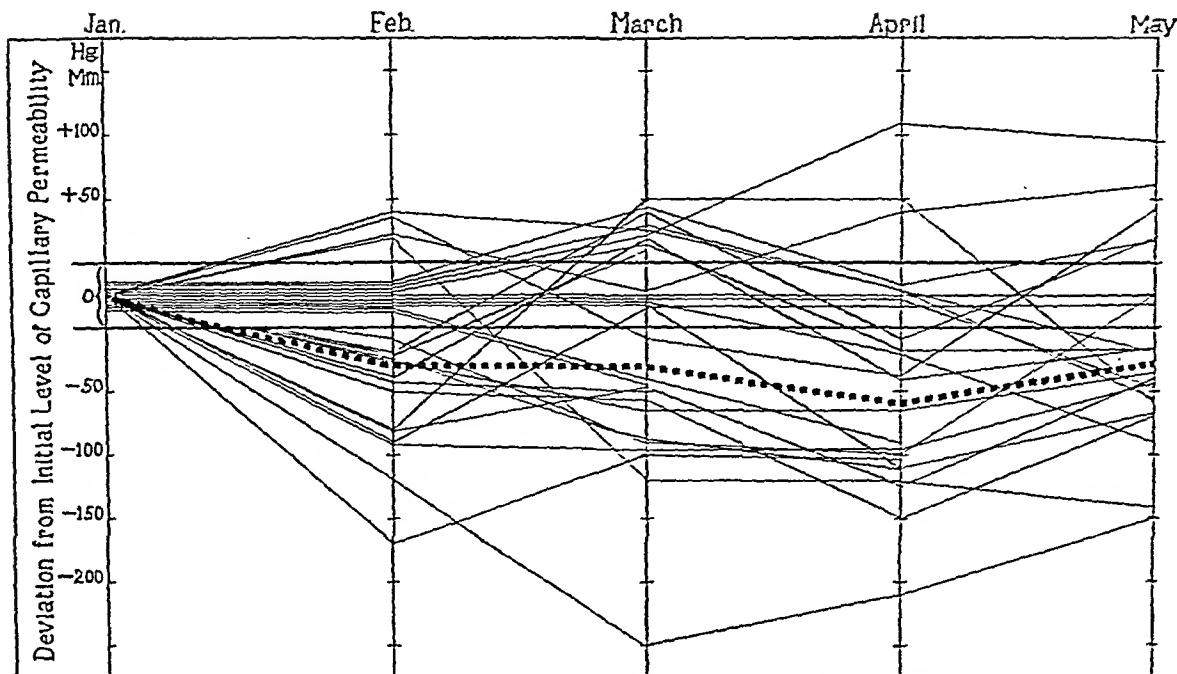


FIG. 2. RELATIVE CHANGE IN CAPILLARY FRAGILITY IN PATIENTS NOT RECEIVING ADDITIONAL ASCORBIC ACID (GROUP B)

Heavy dotted line indicates average for the group.

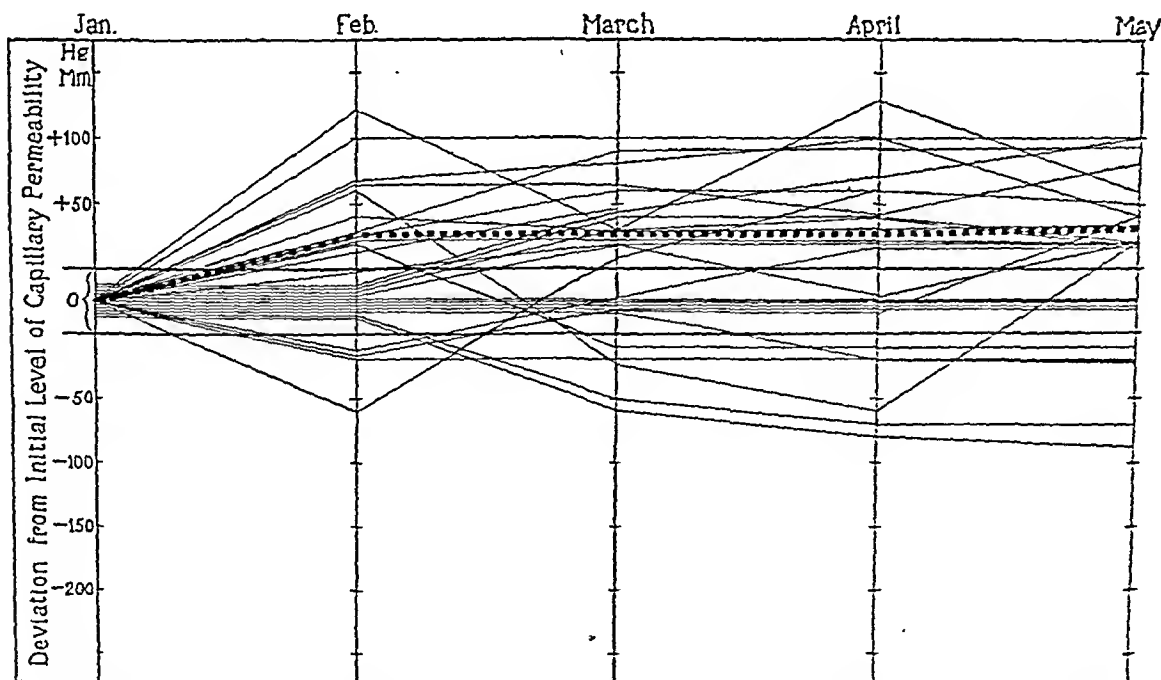


FIG. 3. RELATIVE CHANGE IN CAPILLARY FRAGILITY IN PATIENTS RECEIVING 100 MG. ASCORBIC ACID DAILY (GROUP A)

Heavy dotted line indicates average for the group.

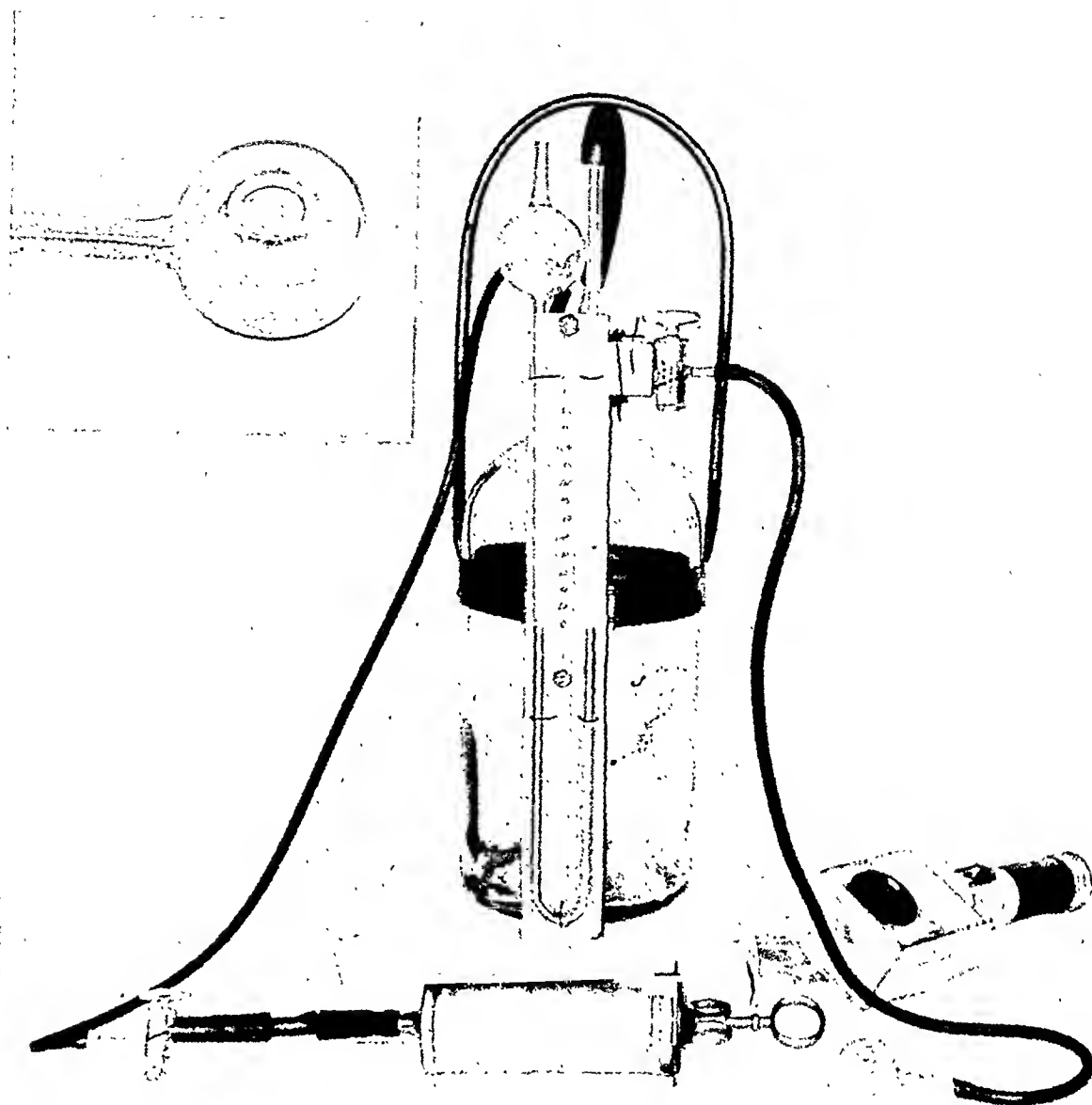


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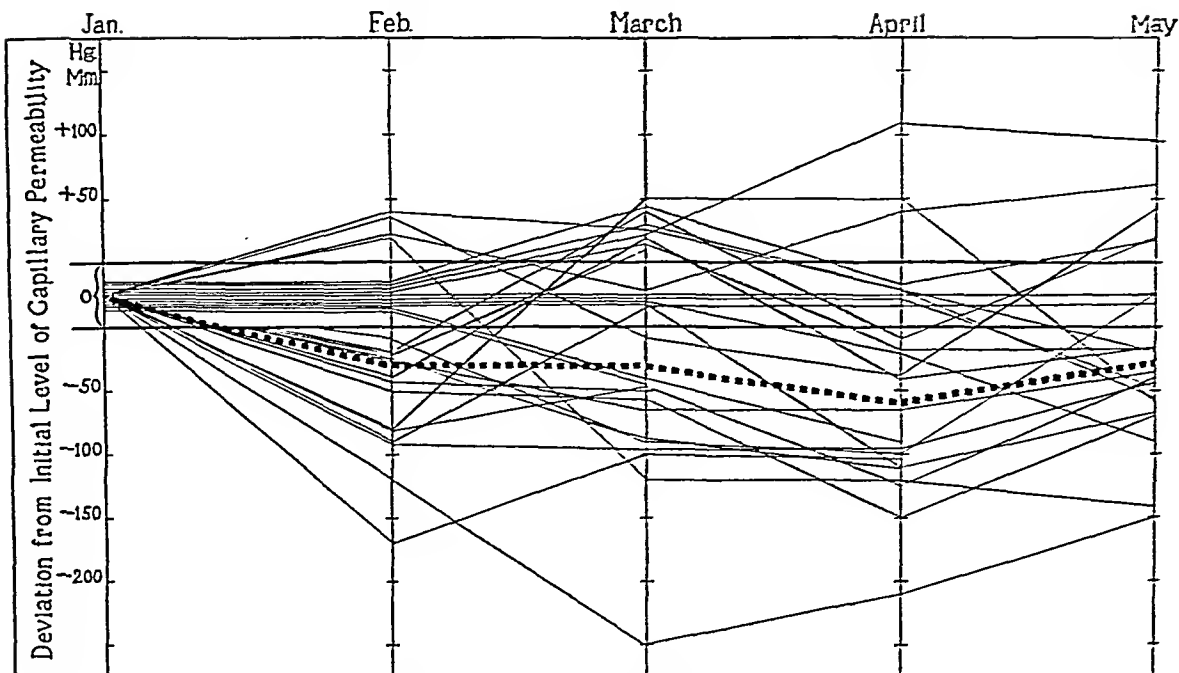


FIG. 2. RELATIVE CHANGE IN CAPILLARY FRAGILITY IN PATIENTS NOT RECEIVING ADDITIONAL ASCORBIC ACID (GROUP B)

Heavy dotted line indicates average for the group.

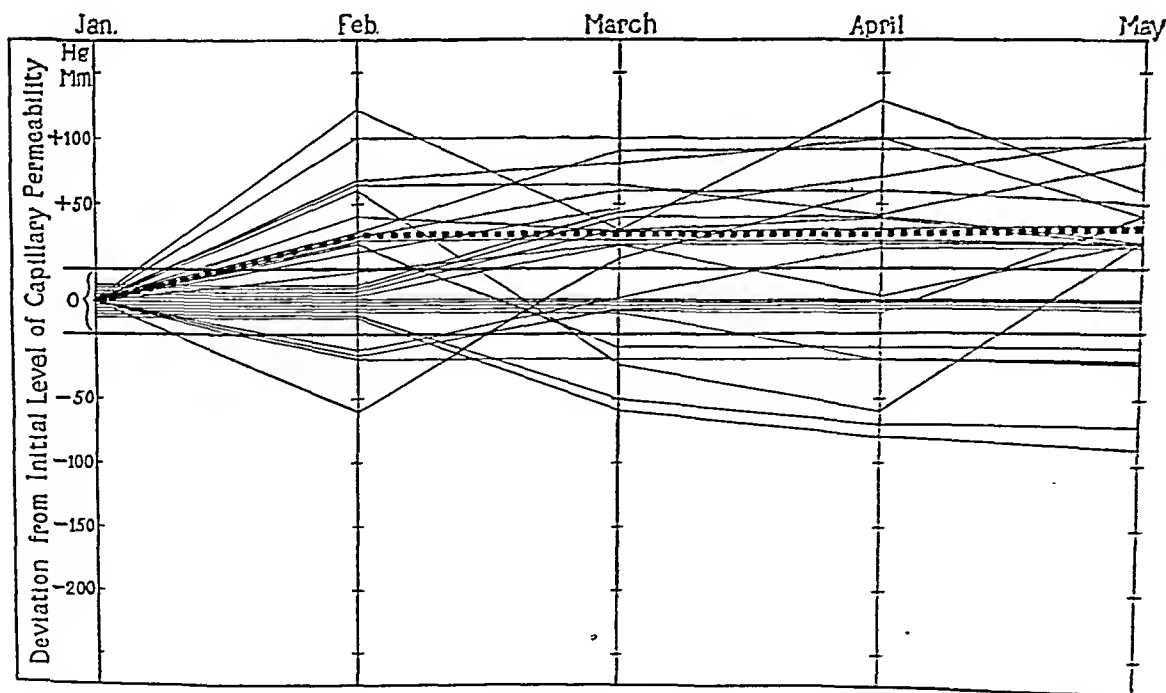


FIG. 3. RELATIVE CHANGE IN CAPILLARY FRAGILITY IN PATIENTS RECEIVING 100 MG. ASCORBIC ACID DAILY (GROUP A)

Heavy dotted line indicates average for the group.

TABLE I  
*Manifestations of rheumatic fever in ambulatory patients*

Case number	Group "A" Treated with ascorbic acid														Group "B" Received lactose									
	1	2	3	4	5	6	8	12	14	17	21	22	23	25	1	5	6	7	8	12	17	20	22	28
Joint and muscular pains.....	+			+		+			+									+						
Chorea.....	+																							
Precordial pain.....		+		+	+	+			+			+					+			+				+
Epistaxis.....				+	+	+	+		+	+	+							+	+					+
Gastro-intestinal symptoms.....			+	+	+	+			+									+	+				+	+
Change in cardiac murmurs.....	+			+	+	+		+	+		+	+						+	+			+	+	
Gallop rhythm.....				+	+	+			+			+	+	+				+					+	+
Erythema multiforme.....						(+)			+			+		+										+
Palpable spleen.....			+	(+)	+					+		+		+										
Pleurisy.....									+		+	+					+							
Leukocytosis.....				+		+			+		+						+			+	+			
Rapid erythrocyte sedimentation rate.....	+			+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+
Fever.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+
Failure to gain weight.....	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+		+	+	+	+	+	+
Tachycardia.....	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+		+	+	+	+	+	+
Summary																								
Acute rheumatic fever.....				+		+			+															
Active rheumatic fever.....	+	+			+			+			+	+	+		+	+		+	+			+	+	
Probably active rheumatic fever			+				+			+				+	+	+	+	+	+		+		+	+

instances of ascorbic acid deficiency were probably present in both groups during January; and furthermore, that, judged by this test, members of Group A were protected against the development of subclinical scurvy, while this condition appeared in several members of Group B. Alterations in degree of capillary permeability could not be correlated with the occurrence of rheumatic activity in these patients. None of them showed any lesions, such as gingivitis, characteristic of scurvy.

#### *Incidence of active rheumatic disease*

Among the 56 subjects investigated, 14 of Group A and 10 of Group B showed evidence of active infection, probably attributable to the presence of rheumatic fever, at some time during the period of observation. The evidence of disease activity is detailed in Table II.

In 4 of each group the symptoms and signs were so slight or so few in number that the designation of "probably rheumatic fever" must be given them. On the other hand, 7 patients in Group A and 6 in Group B had definite evidence of low grade rheumatic activity; while 3 additional members of Group A developed severe acute rheumatic fever during the time they were receiving fairly large amounts of ascorbic acid. The comparative incidence of rheumatic activity among the treated and untreated patients during the various months is shown in Table II. In the

TABLE II  
*Evidence of rheumatic fever in treated and untreated groups*

Number of		January	February	March	April	May
Patients showing some degree of rheumatic activity	Treated with ascorbic acid	9	12	9	6	7
	Given lactose	4	7	7	5	1
Patients with acute rheumatic fever	Treated with ascorbic acid	0	1	1	3	3
	Given lactose	0	0	0	0	0

group receiving ascorbic acid there were twice as many active cases when medication was begun in January as there were in the group receiving the placebo. This greater tendency to develop recurrences doubtless accounts for the higher incidence of rheumatic activity in this group. Nevertheless, if latent scurvy were an important element in calling out relapses it would be probable that measures which are effective in eliminating scurvy would also prevent the rheumatic relapses, but this was not the case. With the exception of a greater relative incidence of recurrences in Group A no differences were apparent in the behavior of the two groups.

#### *The effect of diet*

Even after detailed inquiry it was impossible to grade accurately all of the habitual diets according to their relative ascorbic acid content. A

TABLE III

*Relationship between adequacy of ascorbic acid in diet and occurrence of rheumatic relapses*

Approximate ascorbic acid content of hospital diet	Group "A" in January*		Group "B" during observation		Totals		
	Number of cases	Number with active rheumatism	Number of cases	Number with active rheumatism	Number of cases	Number with active rheumatism	Per cent active rheumatism
Definitely deficient.....	3	2	4	1	7	3	42.8
Intermediate.....	13	4	11	4	24	8	33.3
Adequate.....	12	3	13	5	25	8	31.2
	28	9	28	10	56	19	

\* Before administration of ascorbic acid.

few were identified as definitely deficient in this respect, but almost half of them seemed adequate. The remainder were graded as intermediate. The incidence of active rheumatic disease in these three categories, as indicated in Table III was slightly higher in the individuals taking diets deficient in ascorbic acid. The significance of this correlation is clouded by the fact that these diets were without exception deficient in other respects. Among the patients falling into this "deficient" classification, furthermore, were those living under the most unsatisfactory general hygienic environment.

*The therapeutic effect of ascorbic acid in rheumatic fever.* The therapeutic effect of ascorbic acid medication was tested in 20 hospital patients with rheumatic fever. Three of them had received daily doses of 100 mgm. of ascorbic acid for 1 to 3 months before they were admitted to the wards because of acute illness. All were given ascorbic acid in conjunction with antipyretics when indicated, beginning on the third hospital day and continuing for varying periods of time. Seventeen patients received 250 mgm. of ascorbic acid daily by mouth or intravenously for from 1 to 5 months (average  $2\frac{1}{2}$  months), and 7 (including 4 previously given synthetic ascorbic acid without demonstrable benefit) were fed 200 cc. of orange juice daily for two months. In 11 patients the oral doses of ascorbic acid were replaced by intravenous injections of an equivalent quantity for periods of ten days each during different phases of the disease. Meanwhile, the diets contained liberal quantities of other accessory foodstuffs, or were supplemented by potent yeast and cod liver oil preparations. None of

these measures exerted a demonstrable beneficial influence upon the clinical picture of rheumatic fever. During the course of these treatments there were several instances in which each of the following manifestations of the disease appeared in characteristic fashion: arthritis, carditis, erythema marginatum, subcutaneous fibroid nodules, and prolonged low grade fever. In several patients all other treatment was withheld except a high intake of ascorbic acid combined with adequate caloric diets, and the rheumatic condition progressed, so that eventually the usual antirheumatic therapy had to be instituted in order to relieve the unpleasant symptoms.

#### DISCUSSION

The importance of the disease rheumatic fever warrants a thorough investigation of any valid suggestion concerning possible etiological factors. That made by Rinehart (1933) and his coworkers seemed of sufficient import to excite considerable interest, especially in view of the newer knowledge concerning the structure and action of ascorbic acid. We have, therefore, attempted to apply all possible techniques to the investigation of the relationship between ascorbic acid deficiency and rheumatic fever. Our data concerning the synergic effect of ascorbic acid deficiency and streptococcal infection in guinea pigs, in general agree with Rinehart's, but we differ in our interpretation concerning the resemblance of the lesions so induced with those of human rheumatic fever, and feel that it is too remote to be more than suggestive (Schultz (1936)).

In view of the lack of any suitable subject among the usual laboratory animals for testing this hypothesis further, it seemed to us that the final evidence would have to be obtained from a study of human subjects in whom the disease existed or was likely to occur. We have, therefore, attempted to determine whether the mode of utilization of ascorbic acid was different in rheumatic patients from non-rheumatics, and also whether this substance had any prophylactic or therapeutic influence in this disease. As already noted, the answer to the first of these questions was in the negative insofar as utilization can be determined in terms of intake and output of definite quantities of ascorbic acid (Sendroy and Schultz (1936)). The methods available gave us no evidence con-



cerning the intermediate utilization of this substance, and we can simply state that apparent disturbances in rheumatic subjects follow the same pattern that exists among non-rheumatics. While ascorbic acid subnutrition seemed to occur more frequently among the rheumatics than among the controls, this was by no means a characteristic finding, and when such a state was detected it was attributable to the patient's economic condition rather than to any particular diseased state. When the subjects, both rheumatic and non-rheumatic, received adequate preliminary doses they showed a high degree of saturation with respect to this substance. Although others (Schroeder (1935); Gabbe (1934)) have suggested that in certain diseased states anomalies in ascorbic acid metabolism may occur, definite proof of this has not been presented. In this connection the difficulty of measuring the excretion of the substance in the urine of patients with jaundice should be mentioned; and also the fact that gastro-intestinal disturbances apparently interfere with its absorption from the stomach or bowel. Febrile states or other conditions accompanied by gastro-intestinal upsets may, therefore, apparently affect the excretion of this substance, but when such conditions exist the intravenous administration of ascorbic acid is followed by a normal pattern of excretion.

It was thought that contributory evidence concerning the relationship between subclinical scurvy and rheumatic fever might be obtained by measuring the capillary permeability of the subjects under investigation. Divers states may be accompanied by a decrease in this permeability (Dall-dorf (1933); Cutter and Marquardt (1930); Stephan (1921)); and scurvy has long been recognized as one of them. Particularly through the work of Göthlin and his collaborators the significance of increased capillary fragility as an early manifestation of mild scurvy has been emphasized. The results of applying this test to children in this country have led to different conclusions concerning the prevalence of subclinical scurvy among them (Dall-dorf (1933); Stocking (1933)). Various authors agree that coincident with rheumatic carditis—among other febrile states—capillary permeability is increased (Dall-dorf (1933); Cutter and Marquardt (1930); Stephan (1921)). Frontali (1927) and Sim

(1929–30), on the other hand, found an unaltered permeability in patients with simple polyarthritis uncomplicated with carditis or cardiac decompensation. On the basis of this test our findings indicate the existence of subclinical scurvy in some, but not all, of the rheumatic children studied. It is probable that this increase in permeability was an expression of dietary deficiency rather than of a rheumatic state, because no characteristic change in capillary permeability accompanied the onset or disappearance of signs of rheumatic activity in either group.

The taking of adequate quantities of ascorbic acid over periods of several months did not prevent the recurrence of rheumatic activity among subjects who would otherwise have been expected to develop the disease, even though the results of capillary permeability tests indicated that individuals so treated were removed from a state of subclinical scurvy. This appears to us to be most significant evidence that ascorbic acid deficiency is not an important factor in the etiology of this disease. The failure of large doses adequately taken into the circulation, either enterally or parenterally, to alleviate the symptoms of rheumatic fever, or to prevent the appearance of new symptoms, is further proof in this respect.

It has been recently reported (Euler and Malmberg (1934); Euler, Söder and Malmberg (1935)) that fruit juices contain an "anti-infective vitamin" "J." In addition to pure ascorbic acid, a number of our patients were given large amounts of orange juice and other fruit juices without any appreciable benefit.

Evidence based on the study of the dietary habits of patients in respect to ascorbic acid is, naturally, difficult to evaluate, for a group of persons who have a low intake of this substance probably are in economic conditions where other vitamins and also the total caloric value of their food is subnormal. Faulkner (1935), in a preliminary report of a study of 27 cases of rheumatic fever treated with ascorbic acid, has found large doses of this substance to be without effect on the course of the disease. Warner, Winterton and Clark (1935) have recently investigated in detail the dietary habits of a large group of rheumatic children compared with non-rheumatic controls, and found no significant difference in the two groups with respect to ascorbic acid. We are in agreement

with their conclusions that deficiency of ascorbic acid intake is not a characteristic phenomenon in rheumatic fever, and therefore feel that subclinical scurvy is not a necessary factor in the causation of this disease.

### CONCLUSIONS

1. Two comparable groups of rheumatic children, one of them receiving daily doses of ascorbic acid, were observed at intervals during late winter and early spring. As indicated by tests of capillary permeability, the development of subclinical scurvy was prevented in the treated group, but the incidence of active rheumatic fever was not favorably affected by this medication.

2. The clinical manifestations of acute rheumatic fever were not demonstrably affected by the oral or intravenous administration of ascorbic acid over periods of several months. Large doses of orange juice were also ineffective.

3. These data are additional evidence that ascorbic acid deficiency is not a necessary factor in the etiology of rheumatic fever.

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# A STUDY OF EXTERNAL PANCREATIC SECRETION IN MAN

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An opportunity to study pure pancreatic secretion in man is extremely rare. Wohlgemuth (1) reported his observations in a young man with an external pancreatic fistula. He found that the rate of secretion varied considerably with the diet, that no flow was observed with a high fat diet, that there was an increased rate of secretion with

## Quantitative studies of the composition of the pancreatic fluid

While at the hospital, specimens were obtained daily from the pancreatic fistula. About 15 cc. were obtained during an interval of about 12 hours. The patient was fed various diets as indicated in Table I.

TABLE I  
Quantitative analysis of pancreatic secretion

		CO <sub>2</sub>	Cl	Creat- inine	Uric acid	Urea	Non- protein nitro- gen	Total nitro- gen	Total pro- tein *	Cho- les- terol	Sugar	Cal- cium	Phos- phorus	Pos- ta- sium	Total base	Phos- pha- tase	pH
		vol- umes per cent	mgm. per 100 cc.			mgm. per 100 cc.	mgm. per 100 cc.	mgm. per cent	per cent	mgm. per cent	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mm. eq.	units per 100 cc.	
C250 P 75 F 75	High carbo- hydrate diet to May 3	April 24	382	Traces	Traces	3	28	75	0.29	Neg.	18	3.7	2.2	16.9		12.7	8.6
		April 26	383	Traces	Traces	4	40	137	0.61	Neg.	15	4.1	3.2	21.7		0.9	
C 60 P 60 F200	High fat diet	May 6.....	390	Traces	Traces	3	35	70	0.22	Neg.	9.6	3.5	2.1		162	2.5	
		May 7.....	385	Traces	Traces	2	33	66	0.21		12.5		2.2	17.4	158	8.8	
		May 8.....	391	Traces	Traces	3	30	65	0.22	Neg.			2.2	16.1	170	2.5	
	Full ward diet	May 12.....	62.0	413	Traces	Traces	1	39	0.18		8.5	4.2	2.2	16.9	177	0.8	8.6
		May 13.....	Mixed														
		May 14.....	80.5	380	Traces	Traces	0.5	36	0.18		16.3	3.4	1.9		156	1.1	8.8
Blood serum	May 13.....		66.0	355			16.0	30	1225	7.5	257	94	10.2	3.7	13.9	156	2.8

\* Total protein = 6.25 (total nitrogen-nonprotein nitrogen).

a meat diet and a continuous flow with a carbohydrate diet. Nutt (2) studied a patient with a fistula that remained open for more than a year. There was no lipolytic, moderate proteolytic, and marked diastatic activity. Deutsch and Grubel (3) reported their quantitative study of pancreatic cyst fluid. In 1932, Kahn and Klein (4) observed a patient with an external pancreatic fistula.

We recently observed a woman of 46 with a partial external pancreatic fistula, which had followed the removal of a multilobular cystadenoma of the tail of the pancreas. Quantitative chemical analyses and studies of dye excretion were carried out on the pancreatic fluid.

The results of the chemical analyses are shown in Table I. The last line represents the values found in the blood serum of the same patient.

All of the chemical determinations were carried out in duplicate. The agreement between the duplicates was well within the experimental error of the methods. The accuracy of the technique was also checked by means of known solutions. The urea values are somewhat inaccurate because of the small values found. The phosphatase was controlled by parallel determinations of bone and serum phosphatase of known activity.

## METHODS

Total base was determined by the method of Van Slyke, Hiller and Berthelsen (5), and CO<sub>2</sub>

content according to Van Slyke and Neill (6) using the factors of Van Slyke and Sendroy (7). Chlorides were determined on 0.2 cc. of solution according to the Wilson and Ball's modification (8) of the Van Slyke (9) procedure except that 0.2 cc. of 0.15 N  $\text{AgNO}_3$  was used and 0.01 N KCNS. Urea was determined by the method of Van Slyke and Cullen (10), and uric acid according to Benedict (11). Creatinine was estimated according to the method of Folin and Wu (12). Nonprotein nitrogen was determined by precipitating the proteins with 5 per cent trichloroacetic acid performing a micro-Kjeldahl on an aliquot of filtrate. Howe's method was used for the determination of total protein (13). The Parnas and Wagner modification of Pregl's micro-Kjeldahl method was used for the final determination of nitrogen, except that the  $\text{NH}_3$  was caught in boric acid and titrated with N/100 sulfuric acid (14). Cholesterol was determined by Sackett's modification of Bloor's method (15), calcium by the procedure of Kramer and Tisdall (16), inorganic phosphorus by the method of Fiske and Subbarow (17), and sugar by the Kramer-Gittleman modification of the Folin-Wu method (18). pH was determined in a colorimetric block by the method of Henderson and Palmer (19). Phosphatase estimations were made according to the procedure outlined by Bodansky (20) except that for the final colorimetric determination of phosphate the Fiske-Subbarow method was used. For the determinations on the pancreatic juice the pH of the substrate was adjusted to a pH of 8.8. A phosphatase unit is the amount of phosphatase which will liberate 1 mgm. of inorganic phosphorus in one hour from the buffered glycerophosphate substrate. Potassium was determined by the method of Sobel and Kramer (21).

#### *Study of dye excretion*

Our patient was injected intravenously with 5 cc. of indigo carmine on April 25, 1935, and intramuscularly on May 1, 1935, with 2 cc. of neutral red (2 per cent). These dyes were not perceptible in the pancreatic secretion over a period of two hours.

#### DISCUSSION

The relatively short period of observation did not permit drawing any broad conclusions as to

alterations of composition or rate of flow under the influence of various diets. Daily total excretions could not be determined since our patient had an incomplete external fistula. It is of interest to note that there was a minimal secretion during fasting. Soon after the ingestion of food, there was an increased rate of secretion. It is also significant that the fistulous opening showed no evidence of digestion, owing to the fact that this juice was non-activated.

The results of the serum analyses are typically normal except for the potassium which is below the accepted normal value (22). The chemical analyses of the pancreatic fluid fails to reveal any definite relations to the diet. (See Table I.) The fluctuation in chloride concentrations are minor and are paralleled by a change of total base in the same direction. Both are somewhat above the normal values for blood serum. The potassium values of the fluid are within the range of normal established for serum, while the calcium values in the fluid are less than one-half of the values that are found in serum and are fairly constant. The inorganic phosphate values are also fairly constant, with one exception, and are lower than those of serum. The concentration of sugar is much lower here than in any of the other body fluids. Total protein concentrations with one exception are about the same as those found normally in spinal fluid.

The results of the nonprotein nitrogen determinations in the pancreatic fluid are at the upper end of the normal range for serum and are fairly constant. In view of this the low urea values and the almost negligible uric acid and creatinine values are surprising. The bulk of the nonprotein nitrogen must be different from that of the serum, in which urea, uric acid and creatinine constitute over 50 per cent of the total. Cholesterol is completely absent. This is usually the case for extracellular fluids in the absence of large amounts of proteins. The  $\text{CO}_2$  determinations were usually omitted because the samples were not obtained under oil. The pH of this fluid varied from 8.6 to 8.8. Although these specimens were not taken under oil, it is unlikely that there was an appreciable change in the hydrogen ion concentration.

The phosphatase activity varied considerably in the various samples. The reason for this is obscure. The presence of phosphatase in the pancreatic secretion of man has not been previously reported. Umeno (23) established the presence of phosphatase in pancreatic juice recovered from experimental fistulae in dogs.

The sugar concentrations are in qualitative agreement with those of Nutt. No creatinine or uric acid values are available in the literature for comparison. The urea levels appear to corroborate the findings of J. B. Cohen (24) who observed that in animals the urea concentration of pancreatic fluid is considerably less than that of serum.

Crandall, Oldberg and Ivy (25) injected dogs intravenously with indigo carmine and neutral red. They also reported that these dyes were not recovered in the pancreatic juice. Ingraham and Visscher (26) concluded that all dyes eliminated by the dog's pancreas ionize, with their chromogen electro-negative, under proper conditions. The rapidity of closure of the pancreatic fistula did not permit us to continue further investigations of dye excretion.

#### SUMMARY

1. Quantitative studies of the chemical composition of external pancreatic secretion were made.

2. Injected indigo carmine and neutral red did not appear in the pancreatic secretion.

3. An increased rate of secretion was observed after the ingestion of food.

4. Non-activated pancreatic secretion did not excoriate the fistulous opening.

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# SIMULTANEOUS PLASMA CLEARANCES OF CREATININE AND CERTAIN ORGANIC COMPOUNDS OF IODINE IN RELATION TO HUMAN KIDNEY FUNCTION<sup>1</sup>

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Creatinine is excreted by the human kidney in higher concentration, relative to plasma level, than any other normal urinary constituent. The exceptionally rapid elimination of certain organic compounds of iodine now used in excretion urography suggested that these substances might be excreted even more rapidly than creatinine. The observations described below compare the plasma clearances of urea, creatinine and organic iodine (skiödan, neoskiödan or diodrast, and hippuran<sup>2</sup>) by normal human subjects. Skiödan was cleared from plasma at approximately the same rate as creatinine; neoskiödan and hippuran several times more rapidly than creatinine. Even in the presence of renal disease hippuran clearances were significantly greater than creatinine clearances, without relation to the type of renal lesion.

## PROCEDURE

All subjects omitted breakfast on the day of study and were recumbent during the clearance periods. To a group of ten<sup>3</sup> subjects with normal or almost normal kidney function (Table I) water was given in different amounts to produce a wide range of diuresis. To a second group of seventeen patients with renal insufficiency water was given, with two exceptions (Cases 9 and 11, Table II), at the rate of 200 cc. hourly from three hours before the beginning of the first clearance period to the end of the last clearance period.

Creatinine was administered orally in doses of

3 or 5 grams in the water given one hour before the beginning of the first clearance period. Skiödan and neoskiödan were given intravenously in doses of 13.0 cc. (iodine 2.70 grams) and 20.0 cc. (iodine 3.50 grams) of the respective commercial solutions, approximately fifteen minutes before the beginning of the first clearance period to allow for thorough mixing in the blood stream. Hippuran was administered orally in doses of 12 grams dissolved with the creatinine in the water taken one hour prior to the beginning of the first clearance period.

Urine was collected over two periods, each approximately one hour in duration, and timed to the nearest minute. In order to estimate the percentage of the entire dose eliminated in a given period, the urine formed in the interval between the administration of the organic iodine compound and the beginning of the first period was also saved. Venous blood samples were collected (using lithium oxalate as anti-coagulant) at the beginning and end of each clearance period so that average plasma concentrations of urea, creatinine and iodine could be determined. The blood was centrifuged within ten minutes after it was drawn from the vein to avoid errors arising from diffusion of the organic iodine compound into the erythrocytes while the specimen awaited analysis.

With neoskiödan, the only compound whose properties in this respect were tested in detail, this diffusion is slow since washed human erythrocytes suspended in an isotonic solution of neoskiödan in distilled water were not appreciably hemolyzed after two hours. When neoskiödan was added to whole blood slight loss of iodine from plasma to erythrocytes could be detected by repeated analyses of separated plasma and washed erythrocytes. The change in plasma iodine, corrected for passage of water from cells to serum,

<sup>1</sup> The expenses of this investigation were in large part paid by a grant from the Commonwealth Fund.

<sup>2</sup> Skiödan, sodium mono-iodo methane sulphonate, contains 52 per cent of iodine. Neoskiödan or diodrast, 3:5 di-iodo-4-pyridon-N-acetic acid diethanolamine, contains 50 per cent iodine. Hippuran, sodium ortho-iodo-hippurate, contains 34.4 per cent iodine, when allowance is made for moisture in the powdered substance.

<sup>3</sup> One subject received neoskiödan and hippuran.



TABLE I

*Plasma clearances of urea, creatinine and iodine (in form of skiodan, neoskiodan and hippuran) in patients with little or no renal insufficiency*

Iodine compound given, dose and route	Observation number	Urea clearance	Dosage		Average concentration in plasma			Rate of urine formation	Plasma clearances			Ratio Iodine clearance Creatinine clearance	Total organic iodine excreted	
			Organic iodine	Creatinine	Urea	Creatinine	Iodine		Urea	Creatinine	Iodine		Per cent of dose	Time
		per cent normal	grams	grams	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	cc. per minute	cc. per min- ute	cc. per minute	cc. per min- ute		per cent	min- utes
Skiodan (5.2 grams) 13.0 cc. intravenously	1	89 86	2.70	5.0	8.7 8.4	9.6 7.6	15.3 7.1	2.0 2.3	68 65	130 130	107 114	0.8 0.9	59	124
	2	70 58	2.70	5.0	15.8 15.3	9.5 6.0	13.5 7.5	2.4 3.5	53 43	88 86	100 83	1.1 1.0	60	151
Neoskiodan (diodrast) (7.0 grams) 20.0 cc. intravenously	3	52 49	3.50	3.0	18.4 18.3	5.7 5.4	12.8 2.5	2.0 6.3	39 37	114 108	252 323	2.2 3.0	70	135
	4	109 124	3.50	3.0	17.2 16.2	5.7 5.0	9.7 1.3± *	6.8 11.2	82 93	136 167	327 *	2.4 *	83	133
	5	95 109	3.50	3.0	13.2 14.3	3.3 2.6	6.2 0.9± *	14.3 7.5	72 82	211 182	445 *	2.1 *	87	136
	6	64 71	3.50	3.0	10.0 9.7	4.1 2.5	8.1 1.4± *	7.0 7.3	48 54	108 136	291 *	2.7 *	76	141
	7	64 73	3.50	3.0	17.6 16.4	3.8 3.4	6.5 1.0± *	1.6 1.5	44 47	218 210	315 *	1.4 *	50	144
	8	61 64	3.50	3.0	15.1 14.6	3.8 3.3	7.7 2.2	4.0 2.7	46 48	207 153	522 321	2.5 2.1	78	140
Hippuran 12.0 grams orally	9	95 90	4.13	5.0	15.5 15.3	6.7 5.8	2.9 1.5	2.6 2.7	71 68	142 164	669 476	4.7 2.9	30	177
	10	97 83	4.13	5.0	7.5 7.4	6.6 5.6	1.5 0.8	8.0 4.7	73 62	134 108	486 445	3.6 4.1	49	215
	11	69 60	4.13	5.0	13.3 12.2	7.0 5.6	1.3 0.7	1.7 2.9	48 45	87 74	383 255	4.4 3.5	24	205

\* In four cases (Numbers 4 to 7, inclusive) the plasma iodine of the blood sample taken at the end of the second clearance period was practically zero. Since there was no way of telling at what minute of the period the plasma iodine became zero the average plasma iodine concentration could not be calculated. Clearances were therefore not computed.

amounted to not more than 8 per cent in two hours and to less than 5 per cent in forty minutes.

In studying clearances, separated plasma was used for analyses not only of iodine but also of urea and creatinine. Urea was determined in plasma and urine by the method of Van Slyke and Cullen (1914); creatinine by the method of Folin as described by Holten and Rehberg (1931). Urine and plasma were analyzed for iodine by Leipert's (1933) method with the slight modifications mentioned by Elsom, Bott and Shiels (1936). Skiodan and neoskiodan iodine determinations in plasma were regarded as unreliable

if the average plasma iodine concentration was lower than 2.0 mgm. per cent. In the work with hippuran a refinement of analytical technique (evaporation of the iodine absorbate to small volume before titration) extended this reliability to 0.5 mgm. per cent.

The clearances ( $C$ ) of both iodine and creatinine were calculated in terms of cc. of plasma cleared per minute by the usual equation: urinary concentration ( $U$ ) divided by plasma concentration ( $B$ ) times the volume (in cc.) of urine formed per minute ( $V$ ),  $C = (U \times V) / B$ . Urea clearances were calculated both in terms of cc.

TABLE II

*Plasma clearances of urea, creatinine and hippuran iodine in patients with renal disease*

Case number	Diagnosis	Concentrating power (Addis)	Urea clearance	Average concentration in plasma			Rate of urine formation	Plasma clearances			Ratio Iodine clearance Creatinine clearance	Total organic iodine excreted	
				Urea	Creatinine	Iodine		Urea	Creatinine	Iodine		Per cent of dose	Time
			per cent normal	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	cc. per minute	cc. per minute	cc. per minute	cc. per minute		per cent	minutes
1	Nephrosclerosis, benign	1.024	88 66	9.1 9.0	5.5 5.6	4.9 3.2	5.0 5.8	51 49	94 100	280 259	3.0 2.6	30	183
2	Nephrosclerosis, benign	1.022	68 75	6.4 6.3	4.6 3.4	3.8 2.5	3.3 7.5	51 56	112 109	234 160	2.1 1.5	22	181
3	Nephrosclerosis, benign	1.019	30 27	17.6 17.5	7.2 7.0	4.5 3.1	1.0 3.0	16 20	64 60	169 198	2.7 3.3		
4	Nephrosclerosis, benign	1.019	56 57	12.8 12.5	7.3 8.1	5.1 4.0	1.0 1.6	31 40	87 89	213 215	2.5 2.4	40	180
5	Nephrosclerosis, benign	1.017	28 40	22.0 22.0	11.5 12.0	5.0 4.0	1.4 3.9	18 30	31 46	118 202	3.8 4.4	23	178
6	Nephrosclerosis, malignant	1.011	13 18	39.1 39.3	12.4 11.9	5.7 5.2	0.7 1.6	6 12	7 18	14 44	1.8 2.5	5	187
7	Glomerulonephritis, subacute, no edema	1.018	80 77	10.2 10.2	4.5 4.3	1.3 0.7	3.8 3.6	60 58	106 105	368 298	3.5 2.8	23	186
8	Glomerulonephritis, subacute, no edema	1.018	64 62	15.9 15.8	6.5 6.3	3.2 1.9	4.9 5.7	48 47	95 106	313 325	3.3 3.1	37	180
9	Glomerulonephritis, subacute, nephrosis, no edema	1.031	98 78	9.0 9.0	5.8 4.7	0.7 0.7	9.7 6.6	74 59	127 109	476 380	3.8 3.5	40	286
10	Glomerulonephritis, subacute, nephrotic edema	1.019	45 43	15.4 15.2	6.8 6.2	3.1 2.3	2.4 2.2	34 32	87 83	272 244	3.1 3.0	39	243
11	Glomerulonephritis, subacute, nephrotic edema	1.016	34	19.4	9.9	4.6	7.5	26	49	304	6.2	33	131
12	Glomerulonephritis, chronic	1.012	10 12	65.8 65.6	9.6 10.8	6.6 8.7	0.7 1.4	5 8	10 12	16 19	1.7 1.6	6	187
13	Kidney of pregnancy	1.029	64 65	13.5 13.4	6.7 6.3	2.5 1.9	0.7 2.4	28 49	65 99	327 557	5.0 5.6	41	181
14	Kidney of pregnancy	1.027	71 64	12.1 11.7	7.7 6.7	3.0 1.4	3.4 1.6	54 44	159 135	313 287	2.0 2.1	30	176
15	Kidney of pregnancy	1.022	73 72	17.1 17.1	10.0 8.9	2.8 1.5	2.6 4.0	55 54	95 98	341 358	3.6 3.7	39	191
16	Pyelonephritis	1.020	65 74	12.6 12.5	6.7 6.6	1.9 1.2	2.9 2.9	49 56	100 98	326 494	3.6 5.0	30	204
17	Diabetes insipidus	*1.021+?	69 73	7.7 7.9	5.9 6.9	3.6 2.3	6.2 7.5	52 55	139 135	334 377	3.6 2.3	43	180

\* Partially controlled with pitressin.

per minute and in terms of per cent normal, using for the latter the conventional equations of Van Slyke. For the sake of simplicity concentrations of the three iodine compounds were usually recorded in terms of iodine; such notation, of course, does not affect the clearance figures.

#### OBSERVATIONS

##### *The excretion of skiodan, neoskiodan and hippuran by patients with little or no renal insufficiency*

The plasma clearances of the three organic iodine compounds were compared with simultaneous urea and creatinine clearances in eleven observations on ten subjects whose urea clearances

were 49 per cent of normal or more, and whose creatinine clearances were 74 cc. per minute or more (Table I).

The intravenous injection of skiodan produced considerable pain in the arm. Therefore doses of only 13.0 cc. were administered and but two patients were studied. With creatinine clearances from 86 to 130 cc. per minute in four 1-hour periods skiodan iodine clearances ranged from 83 to 114 cc. per minute. The ratio of skiodan iodine clearance to creatinine clearance ranged from 0.82 to 1.14, averaging 0.95.

Neoskiodan was administered intravenously in doses of 20 cc. (7.0 grams) to six subjects with urea clearances between 49 and 124 per cent of normal. With creatinine clearances between 108

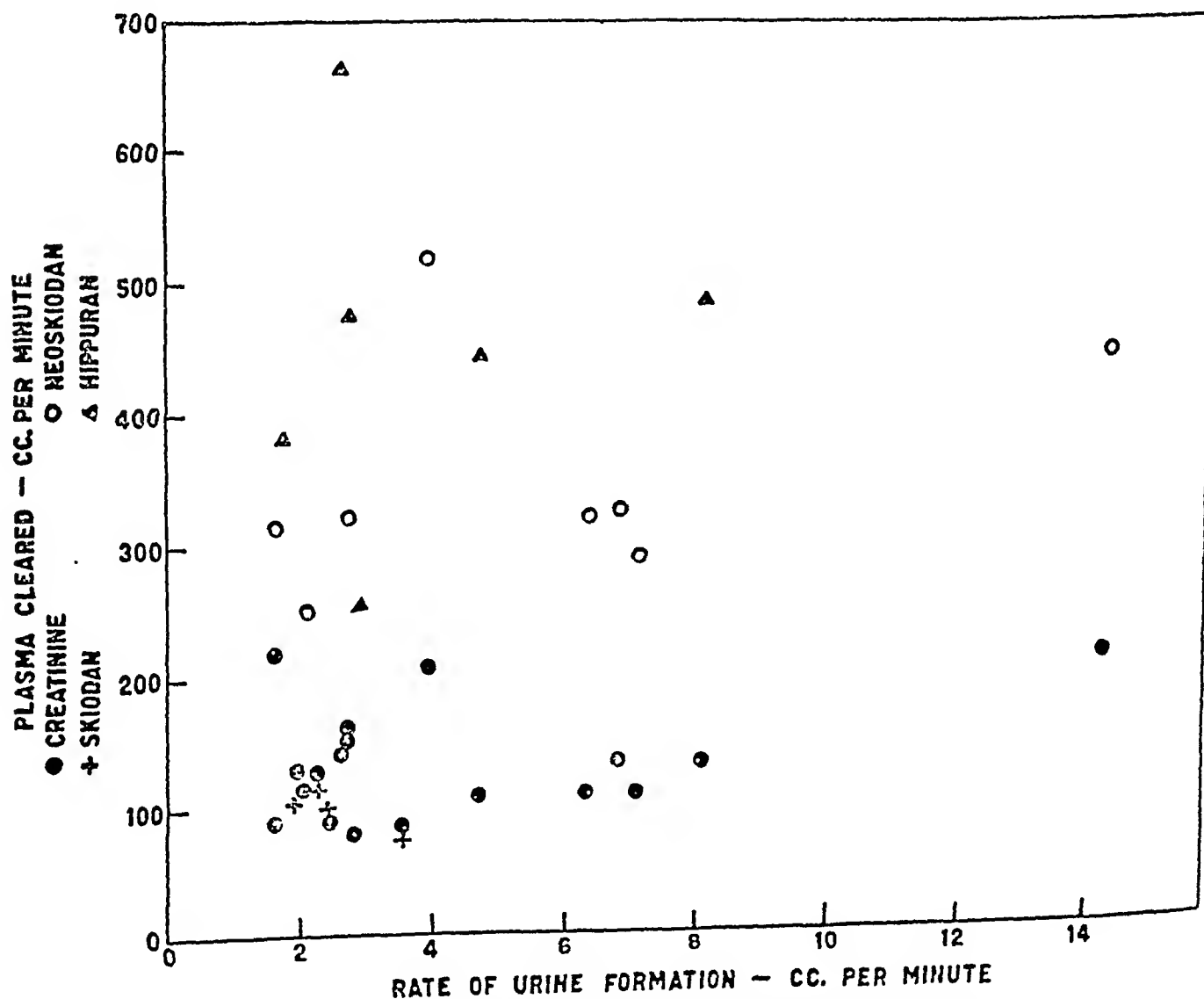


FIG. 1. SHOWING CLEARANCES OF CREATININE (DOTS), SKIODAN (CROSSES), NEOSKIODAN OR DIODRAST (CIRCLES) AND HIPPURAN (TRIANGLES) PLOTTED AGAINST RATES OF URINE FORMATION

and 218 cc. per minute neoskiodan iodine clearances ranged from 252 to 522 cc. per minute. The ratios of neoskiodan clearance to creatinine clearance varied from 1.44 to 2.99, averaging 2.31.

Hippuran was administered in doses of 12.0 grams orally to three subjects with urea clearances of 60 to 97 per cent of normal. With creatinine clearances of 74 to 164 cc. per minute hippuran iodine clearances ranged from 255 to 669 cc. per minute. The ratios of hippuran iodine and crea-

tinine clearance ranged from 2.90 to 4.71, averaging 3.87.

Figure 1 shows that hippuran and neoskiodan clearances were not affected definitely by diuresis ranging from 2 to 14 cc. per minute. Owing to the well known diuretic action of these substances very low rates of urine formation were difficult to obtain even when fluids were restricted. This chart shows also that the lowest neoskiodan and hippuran clearances were greater than the highest

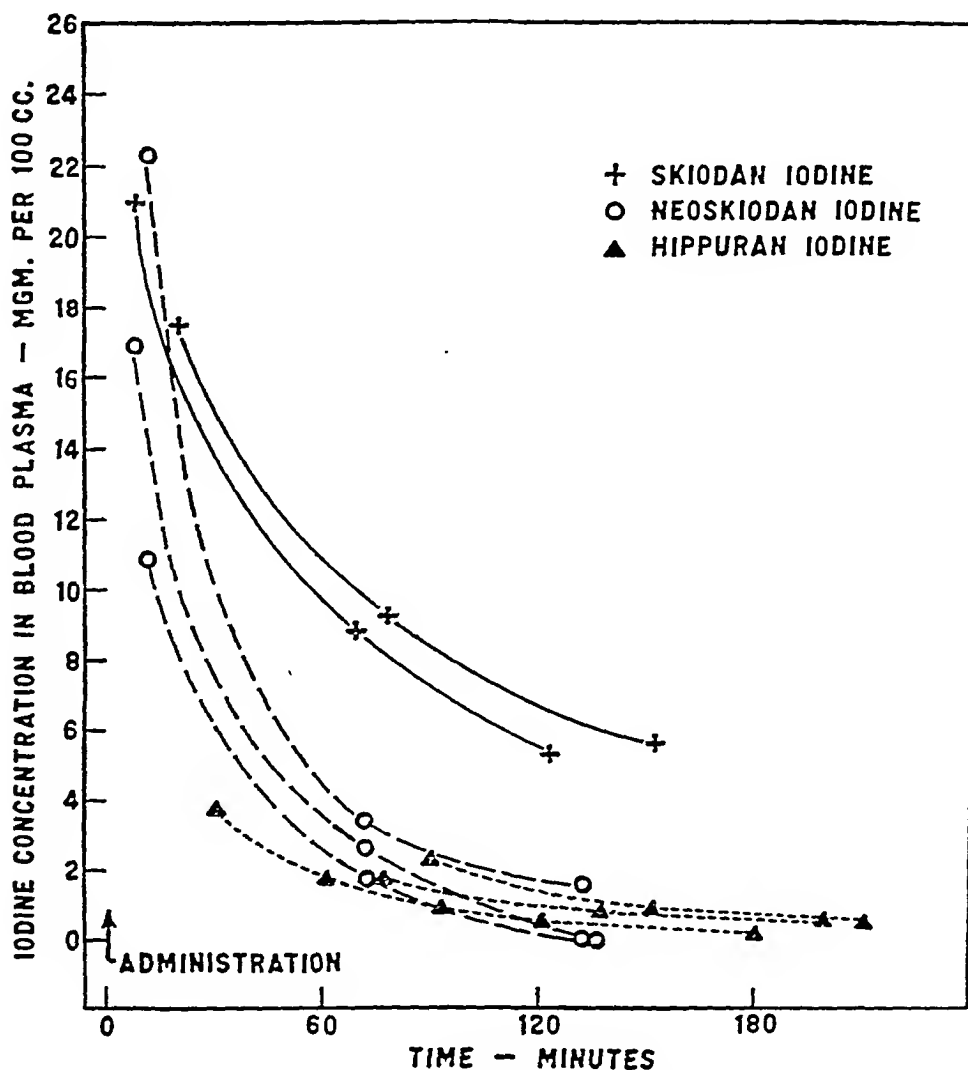


FIG. 2. SHOWING CHARACTERISTIC PLASMA IODINE CONCENTRATIONS DURING CLEARANCE STUDIES ON SKIODAN (CROSSES), NEOSKIODAN (CIRCLES) AND HIPPURAN (TRIANGLES)

Skiodan and neoskiodan were administered intravenously in doses of 13.0 and 20 cc. respectively. Hippuran was given orally in a dose of 12.0 grams.

creatinine clearances. Skiodan clearances, however, were of the same order of magnitude as were the simultaneous creatinine clearances.

The different efficiency with which neoskiodan and skiodan were excreted is reflected by the change in their plasma concentrations shown in Figure 2. These two substances can be compared since both were introduced intravenously. With approximately equal plasma concentrations at the beginning, the plasma concentration of neoskiodan iodine was conspicuously less than that of skiodan iodine at the end of the first hour. Similarly, as indicated to the right in Table I, approximately 60 per cent of the administered skiodan iodine was eliminated by the end of the second clearance period while, with one excep-

tion, 70 per cent or more of neoskiodan iodine was eliminated in approximately the same time.

The plasma concentrations of hippuran and creatinine, after oral administration, are shown in Figure 3 illustrating that hippuran is eliminated far more rapidly than creatinine. In agreement with the clearance figures the changes in plasma concentration (Figures 2 and 3) indicate that neoskiodan and hippuran are both excreted far more efficiently than skiodan and creatinine.

Simple calculation reveals that to explain human plasma clearances of 400 to 500 cc. per minute through glomerular filtration alone would require an almost impossibly great renal blood flow. Observations in man were necessarily limited to smaller harmless doses so that plasma

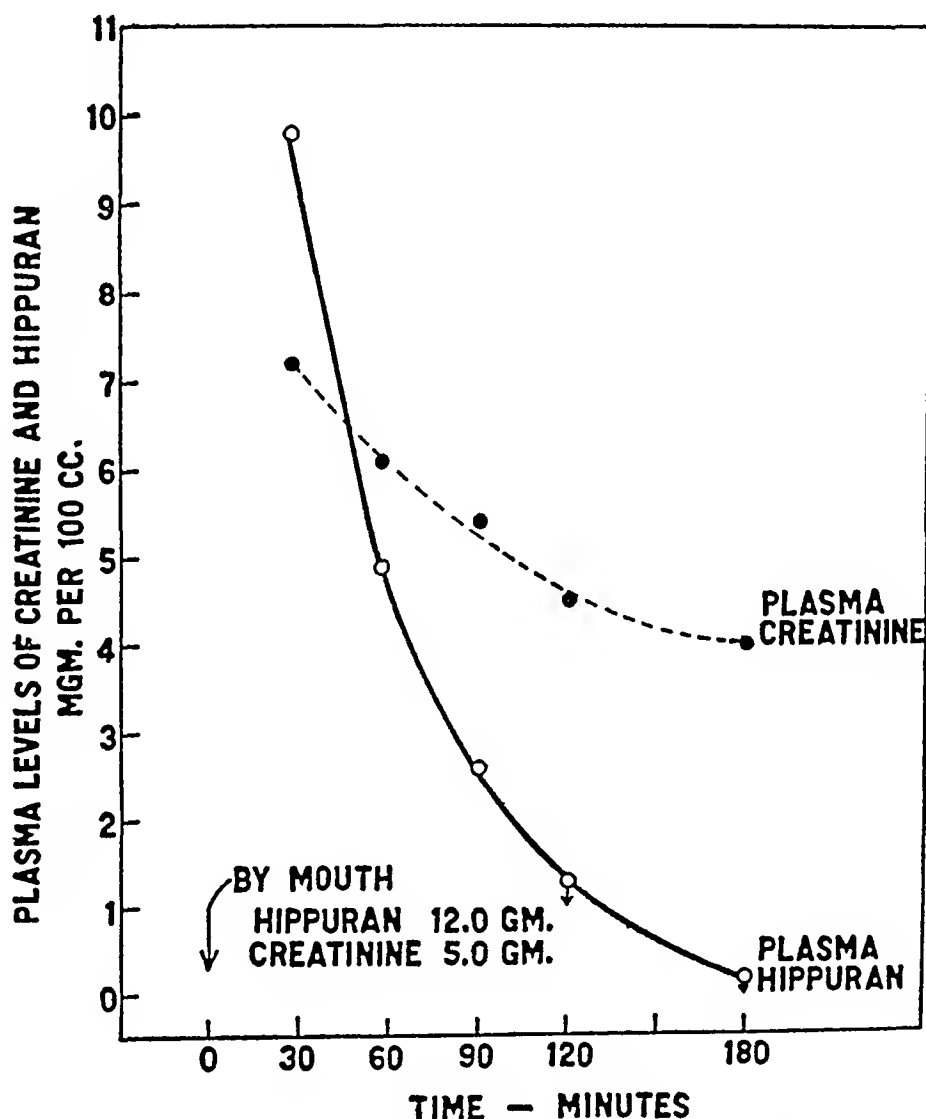


FIG. 3. SHOWING CONCENTRATIONS OF HIPPURAN AND CREATININE IN PLASMA AFTER ORAL ADMINISTRATION OF 12.0 AND 5.0 GRAMS RESPECTIVELY AT ZERO TIME

concentrations of iodine never reached very high levels. In dogs, however, Elsom, Bott and Landis (1934) found that skiodan clearances were approximately equal to creatinine clearances over a much wider range of plasma concentration of iodine (up to 100 mgm. per cent). Neoskiodan and hippuran clearances, while several times as great as creatinine clearances at low plasma iodine concentrations, diminished as the doses of neoskiodan and hippuran were increased. When the concentration of neoskiodan or hippuran iodine in plasma was 70 mgm. per cent or more, their clearances were of the same order of magnitude as creatinine clearances. It was inferred therefore that the high clearances of these two substances at low plasma concentrations must be due to tubular activity. More recent evidence has supported this inference (Elsom et al. (1936)).

*Plasma clearances of creatinine and hippuran in patients with renal disease*

Hippuran was administered to patients with renal disease in order to determine whether a measurable dissociation of glomerular and tubular dysfunction could be detected by comparing hippuran and creatinine clearances. Table II presents data on seventeen cases, grouped primarily according to clinical diagnosis and secondarily in each category according to severity of renal involvement, as measured by a concentration test.

In the course of clinical study quantitative studies of urinary sediment were done, using 12-hour samples of urine collected during complete restriction of fluids for 24 hours (Addis (1925)). In no instance was there any conspicuous diuresis in progress so that even though edema was present (in 2 cases) the restriction in concentrating power agrees in general with diminution in urea and creatinine clearances except in one patient with diabetes insipidus. Urea clearances were determined in the two 1-hour periods with moderate diuresis and also for the 12-hour dehydration period when urine flow was less than 1.0 cc. per minute. For this purpose the 12-hour concentrated urine and a single blood sample taken at the end of the urine collection were analyzed for urea nitrogen, the standard urea clearance being calculated according to the usual equation.

Renal abnormality was ascribed to vascular disease in six cases, to glomerulonephritis in twelve, to pregnancy in three, to pyelonephritis in one and to diabetes insipidus in one. In the first eight cases there was little reason to believe that tubular and glomerular involvement were disproportionate. The remaining nine presented clinical pictures apt to be associated with preponderant abnormality of tubular function and/or structure. It was our purpose primarily to determine whether these two groups excreted hippuran with different efficiency relative to creatinine.

Table II shows, in general, that in each case, irrespective of type or severity of renal damage, hippuran was cleared from the blood plasma from one and one-half to six times more rapidly than creatinine. The total organic iodine excreted in approximately three hours following administration varied considerably. A patient with diabetes insipidus excreted the greatest amount (43.2 per cent) while two patients with isosthenuria excreted 5.4 and 6.1 per cent in the same time. The total elimination showed no constant deviation with small changes in creatinine clearance or concentrating power. It seems doubtful whether studies of total output of organic iodine compounds can be used to detect mild grades of renal insufficiency.

The hippuran iodine clearances (Table II) ranged from 13.5 cc. per minute in a patient with isosthenuria and azotemia to 557 cc. per minute in a patient able to excrete urine with a specific gravity of 1.029. The hippuran iodine clearances were, however, always greater than the corresponding creatinine clearances even when isosthenuria had developed. The relation between the clearances of hippuran iodine and creatinine is shown in Figure 4. The points are distributed approximately about a straight line indicating that with diminishing renal function the creatinine and hippuran iodine clearances are reduced roughly in proportion. The symbols representing the diagnosis in each instance are distributed in a haphazard manner; the points do not group themselves according to diagnosis. It appears therefore that renal insufficiency diminishes the clearances of hippuran iodine and creatinine proportionately, irrespective of the pathology pro-

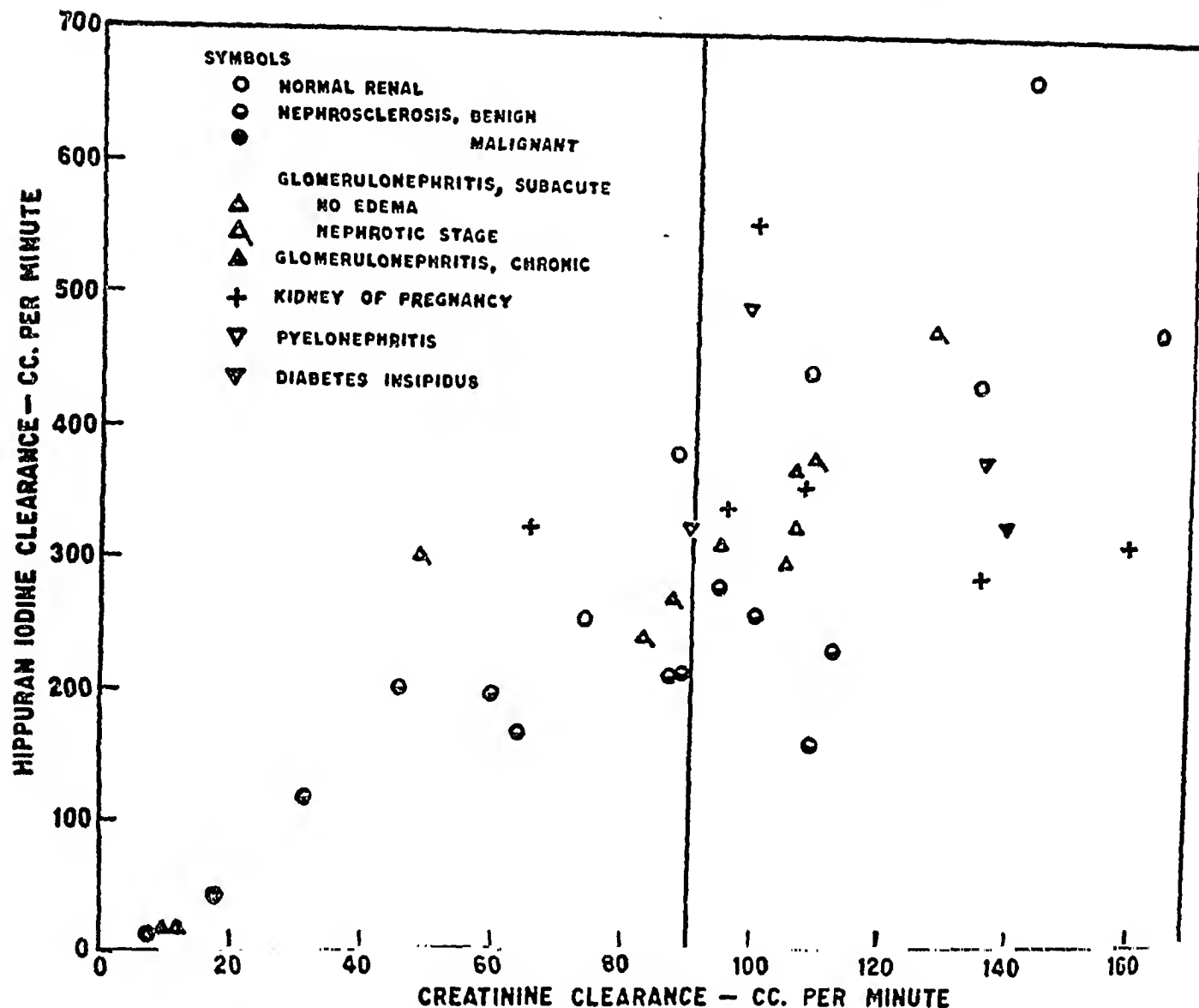


FIG. 4. HIPPURAN IODINE CLEARANCES PLOTTED AGAINST SIMULTANEOUS CREATININE CLEARANCES  
Symbols indicating diagnoses are given in the upper left hand corner.

ducing the insufficiency. If low normal creatinine clearance be taken as 90 cc. per minute hippuran iodine clearances corresponding to normal creatinine clearances range from 160 to 669 cc. per minute. In this group the lowest iodine clearance of 160 cc. per minute was associated with a creatinine clearance of 109 cc. per minute. Patients with nephrosis presented no special deficiency in hippuran excretion.

When hippuran iodine clearances are compared to urea clearances (Figure 5) it is evident that the efficiency with which these two substances are eliminated is likewise reduced proportionately without differences ascribable to the condition producing the reduction in kidney function. If 70 per cent be taken as the lowest normal urea

clearance the hippuran iodine clearances corresponding to normal urea clearance range from 160 to 669 cc. per minute, in complete agreement with the distribution in Figure 4 for creatinine and hippuran clearances.

Hippuran iodine clearances are compared in Figure 6 with the specific gravity of the urine observed in each case during a concentration regime. If a urinary specific gravity of 1.025 be taken as low normal under the conditions imposed, the hippuran iodine clearances in the group designated as normal ranged from 255 to 669 cc. per minute. As in the preceding charts the symbols representing different diagnoses are not segregated so that apparently diminution in hippuran clearance is more closely asso-

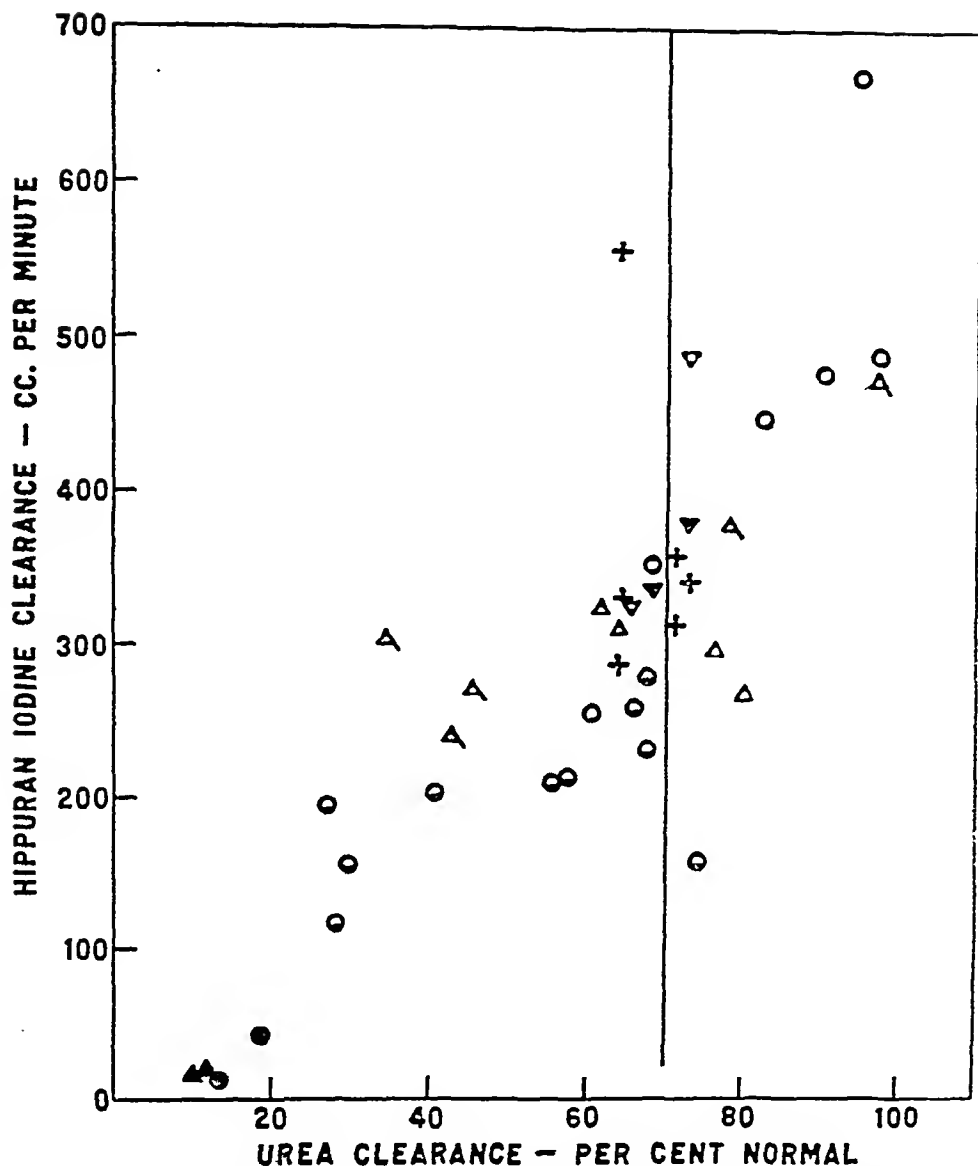


FIG. 5. HIPPURAN IODINE CLEARANCES PLOTTED AGAINST SIMULTANEOUS UREA CLEARANCES. SYMBOLS AS IN FIGURE 4

ciated with restricted concentrating power than with the specific pathology which has produced the renal insufficiency.

Hippuran iodine clearance

The ratio,  $\frac{\text{Hippuran iodine clearance}}{\text{Creatinine clearance}}$ , did not

Creatinine clearance

differ significantly in the various clinical conditions producing renal dysfunction. A ratio of 1.0 would indicate that creatinine and hippuran iodine were cleared at the same rate, while a

ratio of 2.0 or 3.0 indicates that hippuran was removed from the plasma two or three times as efficiently as creatinine. When grouped according to diagnosis these ratios vary widely but are all significantly greater than 1.0, the lowest being 1.47. Ratios found in conditions that are primarily vascular do not differ from those found in conditions that are primarily renal. It is possible, however, that advanced renal damage and isosthenuria lead to progressive reduction in the



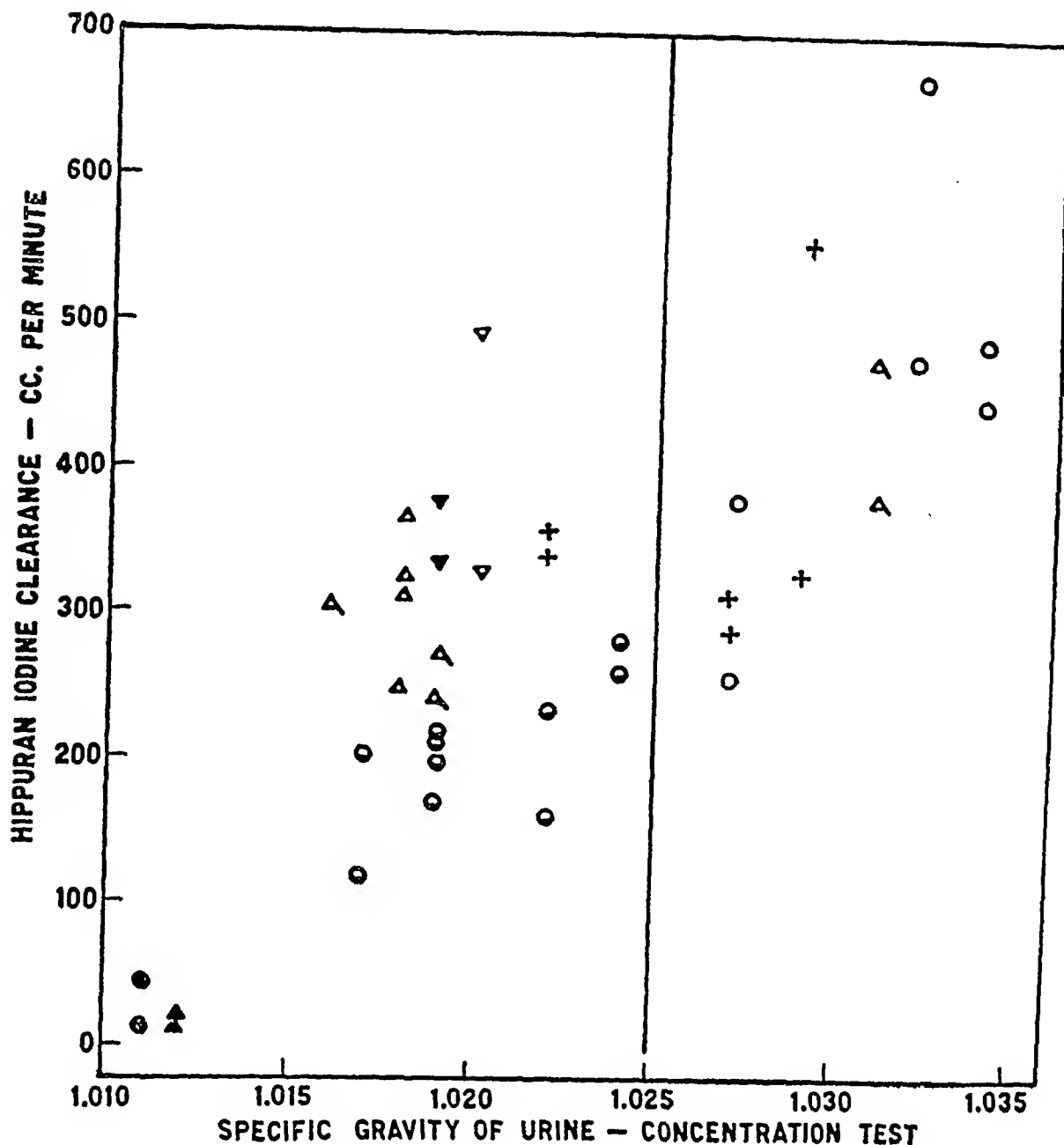


FIG. 6. HIPPURAN IODINE CLEARANCES PLOTTED AGAINST CONCENTRATING POWER EXHIBITED BY EACH PATIENT. SYMBOLS AS IN FIGURE 4

power of the kidney to clear the plasma of hippuran more rapidly than creatinine.

#### DISCUSSION

Recent studies of renal physiology (Richards et al. (1929) et seq.) have massed impressive evidence showing that, in the amphibian, urine is formed through primary ultrafiltration of plasma in the glomeruli, followed by secondary elaboration of this filtrate in the tubules. These studies lead logically to the hope of separating, at least qualitatively, glomerular and tubular components in certain clinical types of renal dysfunction.

Early attempts by Rehberg (1926) to measure glomerular filtrate in man were based on a first assumption that the concentration of creatinine was the same in plasma and glomerular filtrate. This assumption was later proved correct for the amphibian by Bordley, Hendrix and Richards (1933). Second, Rehberg (1926) further assumed that as the glomerular filtrate passed through the tubules creatinine was neither added to, nor lost from, the original glomerular filtrate however much this filtrate might be modified by the reabsorption of water and solutes other than creatinine. The discovery of several substances

which are chemically different from creatinine, but nevertheless are cleared at the same rate as creatinine, would strengthen this second assumption considerably. Richards, Westfall and Bott (1934), and Shannon and Smith (1935) found in dogs that inulin and creatinine were, in fact, cleared from plasma at the same rate. It is interesting to note that in man the clearances of skiodan and creatinine appeared to have the same order of magnitude; however, a larger number of determinations must be performed before it can be concluded that human skiodan and creatinine clearances are identical.

Shannon (1935) observed, however, in normal human subjects that when plasma creatinine is low creatinine clearances are slightly, but definitely, greater than simultaneous inulin clearances. He presented evidence that the human kidney differs from the dog's kidney in that the former appears to eliminate exogenous creatinine partly by tubular secretion. It would therefore be unjustifiable at the present time to assume that creatinine clearance in man is a quantitative measure of the rate of glomerular filtration. It is almost certain, however, that clearances conspicuously greater than those of creatinine must involve tubular activity in addition to glomerular filtration.

Marshall (1931) concluded on this basis that the dog's kidney must eliminate phenol red through tubular secretion. Sheehan and Southworth (1934) state that in anesthetized rabbits filtration alone failed to account wholly for phenol red elimination. By similar reasoning it appears that the human kidney excretes neoskiodan and hippuran partly through tubular secretion. Herbst and Baumrucker (1934) found that neoskiodan and phenolsulfonephthalein when injected intravenously in the same subjects at different times were eliminated at similar, but not identical, rates. In dogs the simultaneous injection of neoskiodan and phenolsulfonephthalein reduced the rapidity with which the latter appeared in the urine. Marshall (1931) calculated that in dogs glomerular filtration accounts for 10 to 17 per cent of phenol red elimination. Our own data indicate that glomerular filtration accounts for 33 to 70 per cent of neoskiodan elimination and for 21 to 34 per cent of hippuran elimination.

Uroselectan has been identified in the glomerular filtrate of frogs after intravenous injection

(Hughes and Peterfi (1931)), and there is no reason to believe that the physical filtrability of the other urographic iodine compounds differs from that of uroselectan. It is far more likely that differences in the rate of excretion depend upon the degree of tubular activity involved. Binz and Maier-Bode (1932) reported that straight chain iodine compounds were eliminated more slowly than a series of iodine compounds containing the benzene ring. Skiodan, the clearances of which were similar to those of creatinine, is a straight chain compound while neoskiodan and hippuran, which are ring compounds, are excreted with plasma clearances several times greater than those of creatinine.

Lichtenberg and Swick (1929) suggested tentatively that the organic compounds of iodine might be of service in estimating kidney function, but artefacts, which make it dangerous to estimate renal function from the intensity of the radiographic shadows produced during intravenous urography, have been emphasized repeatedly (Lichtenberg and Swick (1929); Heckenbach (1930); Lichtenberg (1931); Lindenfeld (1932); Lauber (1932); Olivet (1933); Chabanier and Lobo-Onell (1933); Hefke (1934)). It is generally agreed also that renal insufficiency prevents good radiographic visualization of the urinary tract by means of intravenous urography (Heritage (1930); Jezler (1931); Lichtenberg (1931); Pask (1933), etc.). This clinical observation is explained by the conspicuously diminished iodine clearances in renal insufficiency.

Kidney function has been estimated by studying the percentage of the dose of organic iodine which is excreted in a given time (Heckenbach (1930); Yago (1931); Olivet (1931); Cuthbertson and Jacobs (1932); Lindenfeld (1932) or the specific gravity of the urine after the injection of organic iodine compounds (Heckenbach (1930); Heritage and Ward (1930); Takahashi et al. (1931); Mardersteig (1931 a, b)). Such determinations are subject to error from possible storage in the general body tissues (Olivet (1931); Damm and Junkmann (1932); Lengenmann (1934)) or from variations in the amount of water available for excreting the iodine containing compounds (Heckenbach (1930); Cuthbertson and Jacobs (1932); Damm and Junkmann (1932)). According to our data also,

studies of this type are not likely to detect minor grades of renal insufficiency.

The studies of hippuran clearance in patients with renal conditions were designed to test the possibility of differentiating glomerular and tubular involvement in renal disease. There was no evidence that hippuran clearances provided more information than that obtainable through urea clearances, creatinine clearances or concentration tests. The elimination of hippuran appears to be conditioned by general renal function rather than by specific glomerular or tubular dysfunction. Hippuran clearances, therefore, while of considerable physiological interest, present no clinical advantages to compensate for the greater labor of the analyses involved.

#### SUMMARY

The clearances of three organic compounds of iodine were compared with simultaneous creatinine clearances. In normal subjects skioldan and creatinine clearances were of the same order of magnitude.

Neoskioldan and hippuran clearances were significantly greater than creatinine clearances; the differences were so conspicuous that the elimination of these two iodine compounds can not be explained by simple glomerular filtration, but must be ascribed in part to tubular activity.

In renal insufficiency hippuran clearances were in general reduced proportionately to urea clearances, creatinine clearances and concentrating power. Hippuran clearances were related more closely to the grade of renal insufficiency as a whole than to preponderance of glomerular or tubular dysfunction; they remained greater than creatinine clearances even in advanced renal failure.

The plasma clearances of these organic compounds of iodine, while of considerable physiological interest, provide no special information to recommend their use in the diagnosis or clinical study of renal disease.

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# THE NITROGEN METABOLISM IN ANEMIA DURING THE REGENERATION OF BLOOD

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The present study was made following certain observations upon the plasma proteins in anemia. An attempt was made to explain the initial gain in weight (1) and the appearance of palpable edema in certain anemic patients as their blood improved. It was assumed that the increased need for protein substances in the building of new hemoglobin might result in a reduction of the plasma protein and of the plasma oncotic pressure when the diet contained a reduced amount of protein. No correlation between a reduction of the plasma protein and the appearance of palpable edema, however, was found, perhaps in part due to the fact that gains in weight and the development of edema did not occur to any considerable degree in the patients studied.

Marked changes in the nitrogen metabolism, however, have accompanied the regeneration of blood following the administration of effective material in various forms of anemia in this clinic. These changes manifest themselves as a reduction of the nonprotein nitrogen, plasma protein and nitrogen elimination. That this should be so was not remarkable in view of the considerable demand for protein for hemoglobin formation (for a gain of each 10 per cent in hemoglobin about 80 grams of protein are required). The anemic patient while regenerating hemoglobin, therefore, offered an opportunity to study the effect of sudden protein demand on the nitrogen metabolism of the organism. In addition to the theoretical information to be obtained from the study, it was hoped that points of therapeutic interest might emerge. In the present communication the effect of hemoglobin regeneration on nitrogen retention, on the plasma protein and nonprotein nitrogen, and on the tissue nitrogen is reported. In a subsequent communication the observations will be extended to variations in the remaining nitrogen partitives.

Nine severely anemic patients were chosen for

study. The sex, age of these patients, together with the diagnoses of their cases, are given at the end of the communication. Regeneration of blood occurred during the period of observation in five patients with hypochromic anemia (Cases 1, 2, 4, 7 and 8) when iron was administered; in two patients with pernicious anemia (Cases 5 and 6) following administration of material specifically active in this condition; in one patient with scurvy (Case 9) following the administration of an adequate diet. Case 3, a patient suffering from hypochromic anemia, was given no medication during the period of observation and was used as a control. In addition to this case, the first twelve-day period of Case 7 was used as a control period, and no medication was given during this time.

The patients were given diets which were adequate in calories but were usually low in protein. Water and table salt were unrestricted. Urine was collected for three-day periods. In two instances stools were collected for three-day periods. Venous blood was collected usually every other day. Red blood cell counts and cell volume measurements were made upon this blood. The hemoglobin was measured by a Sahli instrument calibrated as described below. Reticulocytes were counted daily upon smears stained supravitaly with brilliant cresyl blue. The blood volume was measured, usually every fourteen days, by means of the vital red technique as described by Rowntree, Brown and Roth (2).

All chemical analyses were made in duplicate. The plasma proteins were determined upon oxalated blood plasma by the Kjeldahl method, using the macro modification of Howe (3). Certain essential changes in the method were made as described by Peters and Van Slyke (4). The most important of these modifications are as follows: digestion of the plasma was accomplished by the use of 10 ml. of concentrated sulphuric acid

low in nitrogen and 5 grams of a digestion mixture prepared by mixing 500 grams of potassium sulphate and 10 grams of powdered copper sulphate. Complete digestion was assured by the use of Merck's superoxol. Two hundredth normal hydrochloric acid and sodium hydroxide were employed in the titrations. Instead of methyl red, Tashirio's indicator was used in the titrations because of the sharper end-point obtained with acids and alkalis of the dilution used. The indicator was prepared following the suggestions of Kerr and Blish (5).

The nonprotein nitrogen and the urinary nitrogen were determined by the micro-chemical methods of Folin (6). Stools were collected in sulphuric acid, mixed to a homogeneous suspension, weighed, and aliquot samples taken for determination of nitrogen by the usual Kjeldahl method. The average daily stool nitrogen of Cases 3 and 8 and of two additional cases of anemia, not included in this series, was 0.7 gram. This figure was employed in calculating the nitrogen output in those cases in which fecal nitrogen was not determined. This seemed to be a fairer basis of calculation in this particular study than the normal range, which was found to be from 1.25 to 1.50 grams per day.

The plasma protein per cent, blood volume and hematocrit being known, the total circulating plasma protein or plasma nitrogen may be easily calculated. Because of the large number of chemical analyses and the considerable amount of blood which was drawn, analysis of circulating whole blood nitrogen was not made but was calculated from the hemoglobin readings and plasma protein values.

Sahli hemoglobinometers were calibrated in such a manner, employing blood upon which the oxygen capacity was known, that 100 per cent hemoglobin corresponded to an oxygen capacity of 20.9 volumes per 100 ml. of blood, and this was assumed to be the equivalent of 15.6 grams of hemoglobin per 100 ml. of blood.

Determinations of the nitrogen content of washed red blood cells and of hemolyzed red blood cells, free of plasma and stroma, were compared with the amount of hemoglobin ascertained from the Van Slyke oxygen capacity method. Nitrogen values were multiplied by 6.25 to obtain

values for total protein (hemoglobin). In one normal individual and four patients with pernicious anemia the protein equivalents of hemolyzed red blood cells free of plasma and stroma, per 21 volumes of oxygen per cent, were respectively, 15.7, 15.7, 15.8, 15.0, 15.6 grams per 100 ml., or an average of 15.6 grams per 100 ml. of blood. The determination of the hemoglobin nitrogen, therefore, gave results which were quite in keeping with the assumed weight of hemoglobin. The error introduced in determining total circulating nitrogen by not including the nitrogen of the stroma of the red blood cells and of the other formed elements of the blood was small and was disregarded in the present calculation. There is some evidence that the structure of the hemoglobin molecule may vary in different forms of anemia (7), but there is no evidence, as far as the authors can discover, that the nitrogen content or actual size of the hemoglobin molecule varies appreciably.

Since in Cases 7 and 8 there occurred losses of blood in the stools, it is important to consider what effect, if any, such losses have on the experimental findings. In all of the patients in whom loss of blood was responsible for the anemia, except Case 1, the loss of blood occurred in the upper gastro-intestinal tract. Under these circumstances it was considered probable that the protein and other nitrogenous constituents so lost from the blood stream were absorbed and utilized in the same manner as food protein. Such a state of affairs would occasion little loss of nitrogen to the organism. This assumption has been borne out by experimental data. It has been found from other studies that the characteristic low nitrogen content of the stools was maintained even in the face of chronic loss of blood from the upper gastro-intestinal tract. Carefully controlled experiments have shown no increased loss of nitrogen in the stools or in the distribution of the various nitrogenous partitives in the whole blood, blood plasma or urine even when the stools gave a markedly positive test for occult blood. Case 8, for example, was bleeding throughout the experiment from a cancer of the stomach, yet the daily output of nitrogen in the stools (0.7 gram) was in close agreement with that found in Case 6 in which there was no blood loss.

## EXPERIMENTAL

The summary of the experimental results are given in Tables I and II. Table II is included for the purpose of giving the experimental data on which Table I is based. In Table III and Figure 1 the results in a typical case are given. The results for Case 1 in Table I indicate gain or loss of circulating nitrogen somewhat higher than that which may be calculated from Table III. This is because the loss of nitrogen by venesection in Case 1 has been taken into account in the preparation of Table I and appears as manufactured plasma and hemoglobin nitrogen. Inspection of the data in Tables I and II shows that hemoglobin was always formed in response to antianemic therapy, regardless of the extent or nature of nitrogen losses from the organism as a whole. The demand for nitrogen occasioned by this response was met in various ways, depending upon first, the level of the protein in the diet, and second the nutritional state of the patient.

When the nitrogen intake was below 6.2 grams per day, hemoglobin was manufactured at the expense of either the plasma nitrogen, the tissue nitrogen or both. If, however, the amount of nitrogen fed was above 6.2 grams per day, much of the required nitrogen was provided by the diet. *Hemoglobin was always formed in response to therapy even in the presence of tissue and plasma nitrogen deprivation.*

Cases 1, 4, 7 and 8 all show similar responses to nitrogen demand for hemoglobin regeneration and therefore may be conveniently discussed together. In all four cases the nitrogen intake was initially below 6.2 grams per day, and there was a slightly negative nitrogen balance of between 1 and 2 grams per day. It must be borne in mind, however, that this negative balance does not mean that the body was failing to minimize the loss of nitrogen in the urine during nitrogen demand. On the contrary, the daily loss of nitrogen in the urine in some of these cases was below the value generally

TABLE I

*Summary of nitrogen metabolism in anemia during hemoglobin regeneration*

Case number	Diagnosis	Therapy	Period	Number of days	Average daily nitrogen intake	Total nitrogen intake	Total nitrogen output	Nitrogen balance	Total gain of nitrogen in plasma	Total gain of nitrogen in hemoglobin	Total gain of nitrogen in circulation	Total gain of nitrogen in tissues
1.	Hypochromic anemia, chronic loss of blood (menorrhagia), hypochlorhydria.	Iron	1	24	grams 4.0	grams 95.9	grams 123.6	grams -27.7	grams -10.7	grams +22.1	grams +11.4	grams -32.1
		Iron	2	12	18.6	222.8	101.6	+121.2	+6.8	+21.4	+23.2	+93.0
			1 and 2	Total	8.9	318.7	225.2	+93.5	-3.9	+43.5	+39.6	+53.9
2.	Hypochromic anemia, duodenal ulcer, loss of blood.	Iron		27	7.3	195.9	174.8	+21.1	+1.0	+30.3	+31.3	-10.2
3.	Hypochromic anemia, duodenal ulcer, loss of blood.	None		24	9.9	237.4	224.6	+12.8	-0.5	+2.2	+1.7	+11.1
4.	Hypochromic anemia, duodenal ulcer, loss of blood.	Iron		39	6.1	232.6	277.2	-44.6	-22.5	+47.2	+24.7	-67.3
5.	Pernicious anemia.	Potent substance		39	6.4	247.7	231.2	+16.5	-2.1	+50.4	+48.3	-31.8
6.	Pernicious anemia.	Potent substance	1	57	6.5	373.5	321.2	+52.3	-6.5	+46.1	+37.6	+12.7
		Potent substance	2	27	21.1	501.3	237.0	+262.3	-1.3	+20.8	+19.5	+242.8
			1 and 2	Total	11.6	874.8	560.2	+314.6	-7.8	+66.9	+59.1	+255.5
7.	Hypochromic anemia, inoperable cancer of stomach, chronic loss of blood.	None	1	12	3.6	43.5	57.3	-13.8	-0.1	-0.8	-0.9	-12.9
		Iron	2	39	4.6	178.1	193.0	-14.9	-4.0	+27.5	+23.5	-2.4
		Iron	3	24	9.0	215.5	137.1	+78.4	+1.7	+4.3	+6.0	+72.4
			1, 2 and 3	Total	5.8	437.1	387.4	+49.7	-2.4	+31.0	+28.6	+31.1
8.	Hypochromic anemia, inoperable cancer of stomach, chronic loss of blood.	Iron	1	33	3.6	118.5	155.2	-36.7	-3.6	+24.7	+21.1	-57.8
		Iron	2	15	8.3	124.1	69.5	+54.6	-1.7	-8.4	-10.1	+64.7
			1 and 2	Total	5.1	242.6	244.7	+17.9	-5.3	+16.3	+11.0	+6.9
9.	Scurvy, nutritional anemia.	Diet	1	24	6.3	151.5	70.4	+81.1	-3.0	+24.8	+21.8	+59.3
		Diet	2	27	19.0	514.1	214.9	+299.2	+7.5	+34.9	+42.4	+256.8
			1 and 2	Total	13.1	665.6	285.3	+380.3	+4.5	+59.7	+64.2	+316.1



TABLE II

Initial and final values employed in the determination of the total circulating nitrogen

Case number	Hemoglobin		Plasma protein		Blood volume		Plasma volume		Blood lost by venesection
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	
	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	grams per 100 ml.	ml.	ml.	ml.	ml.	ml.
1. Ly.....	4.8	10.8	7.15	7.36	4,000	4,090	3,240	2,580	325
2. Kn.....	8.9	12.0	5.54	5.48	3,340	3,880	2,400	2,300	200
3. Su.....	6.9	7.5	6.05	6.40	5,140	4,690	3,930	3,540	180
4. La.....	5.6	11.5	7.00	6.13	6,230	5,420	4,900	2,760	350
5. Maz.....	6.2	11.9	5.72	6.04	4,520	4,930	3,699	3,060	280
6. Mac.....	5.5	14.5	6.21	5.65	3,840	3,880	3,220	2,160	740
7. Da.....	7.6	11.7	6.02	5.70	3,550	3,440	2,680	2,130	640
8. Be.....	5.5	6.8	5.82	4.89	3,620	3,570	2,880	2,550	355
9. Ha.....	9.4	15.1	5.41	6.60	3,760	4,360	2,730	2,380	500

accepted as the irreducible nitrogen elimination occasioned by normal tissue catabolism and basal metabolic processes. Actually these patients show for long periods of time a considerable reduction of the nitrogen output in the urine which can persist for weeks after the blood has returned to normal. Thus, one of the first responses of hemoglobin demand in the cases investigated is a diminution of nitrogen constituents of the urine.

In the four cases under discussion nitrogen

TABLE III

Case 1. (Ly.) Chronic loss of blood (menorrhagia). Hypochlorhydria

Days	Remarks	Hemoglobin	Nitrogen in circulation	Average daily nitrogen intake	Average daily nitrogen output	Plasma protein
Period 1 (Low nitrogen intake)						
		per cent	grams	grams	grams	grams per 100 ml.
3	Ferrous sulphate 0.8 gram daily.	31	68	4.0	4.3	7.15
6	Ferrous sulphate 1.2 grams daily.	36	71	3.4	5.0	7.73
9	Ferrous sulphate 1.2 grams daily.	38	66	4.0	4.4	6.93
12	Ferrous sulphate 1.6 grams daily.			4.0	5.1	
15	Ferrous sulphate 1.6 grams daily.	46	72	4.0	5.3	7.31
18	Ferrous sulphate 1.6 grams daily.	51	77	5.2	4.4	7.11
21	Ferrous sulphate 1.6 grams daily.	56	75	4.0	6.0	6.55
24	Ferrous sulphate 1.6 grams daily.	59	75	3.4	6.9	6.69
Period 2 (High nitrogen intake)						
27	Ferrous sulphate 1.6 grams daily.	64	84	18.2	8.3	6.63
30	Ferrous sulphate 1.6 grams daily.	60	87	17.8	6.7	6.47
33	Ferrous sulphate 1.6 grams daily.	66	96	19.2	10.2	7.26
36	Discharged. High protein diet.	69	101	19.1	8.7	7.36
49	Ferrous sulphate 1.6 grams daily.	77	107			7.14

spared from urinary excretion was not sufficient to satisfy the demands of the hemoglobin building centers. Instead of the production of hemoglobin

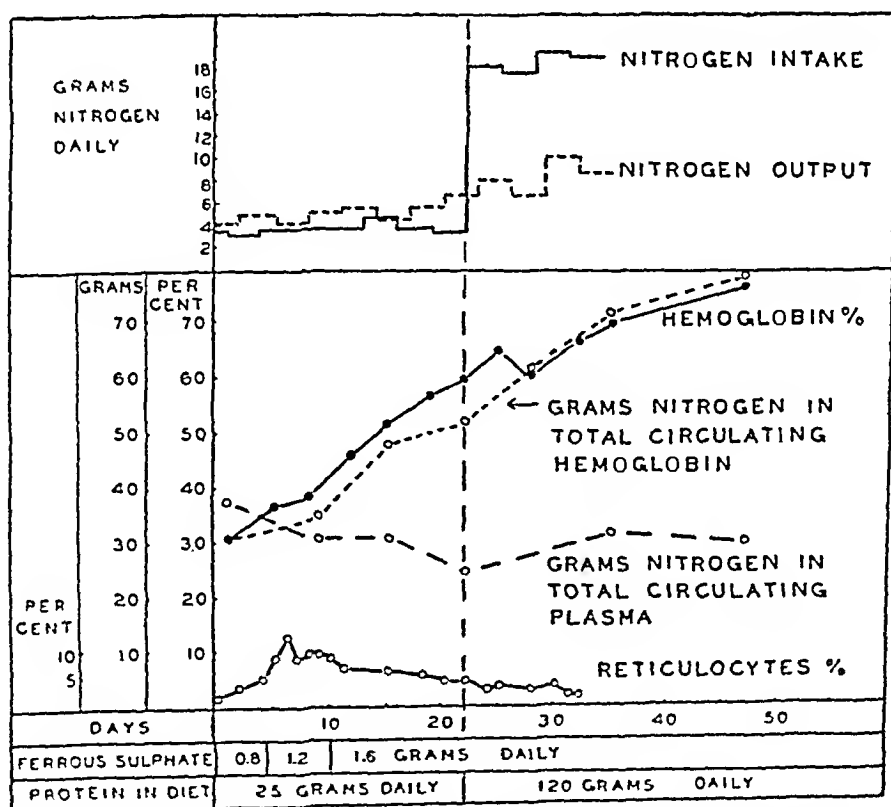


FIG. 1. CASE 1 (Ly.). THE NITROGEN BALANCE AND THE NITROGEN OF THE CIRCULATION DURING HEMOGLOBIN REGENERATION

being slowed under these circumstances, as was at first expected, it proceeded at the same normal rate (about 1 gram of hemoglobin nitrogen per day). *The nitrogen required for this purpose was furnished by the nitrogen of the plasma protein and of the tissues.* For example, there was usually a fall in the total circulating plasma nitrogen as the hemoglobin rose. This is strikingly illustrated by Case 1 (see Figure 1, and Tables I and III). For the sake of brevity the corresponding tables and figures for the other three cases are not given but data may be calculated from that given in Tables I and II.

In three of the four cases under discussion (Cases 1, 7, 8) the period of low protein diet was followed by one of normal or even high protein diet. The results confirmed the findings that there had been marked deprivation of tissue nitrogen during the period of low protein intake and hemoglobin regeneration. With the increase of nitrogen in the diet there occurred a marked nitrogen retention. Coincident with this nitrogen retention the drain on the nitrogen of the circulating plasma and of the tissues ceased. Of the four cases, Case 1 is probably the most significant in this regard since the increase in nitrogen intake took place at a time when the hemoglobin was still being formed at a rapid rate. It will be observed from Table III and Figure 1 that with the increase in nitrogen intake the patient was able to manufacture hemoglobin and at the same time slowly to replace the nitrogen deficit in the circulating plasma.

The limiting condition between the first type of response to hemoglobin formation and the type of response in the remaining cases seems to be the level of the protein intake. If the nitrogen intake was below 6.2 grams per day a positive nitrogen balance never occurred in patients with anemia during their response to antianemic material. The amount of nitrogen fed two patients (Cases 2 and 5) was above this critical level, namely, 7.3 and 6.4 grams, respectively. The effect of hemoglobin regeneration on the nitrogen metabolism of these two patients differed in degree rather than in kind from the patients of the first group. In the first group, with a negative nitrogen balance, the tissue protein and plasma nitrogen satisfied the demand for nitrogen occasioned by hemoglobin formation; in Cases 2 and 5 a portion of the nitro-

gen was definitely provided for by the increased retention of nitrogen, and the tissue and plasma nitrogen were to some extent spared. This is well illustrated in a comparison of Case 4 with Case 5. In Case 4, 47 grams of hemoglobin nitrogen were produced in 39 days in the face of a negative nitrogen balance of 45 grams. To provide the required nitrogen the tissues contributed 69 grams and the plasma 22.5 grams. In Case 5 over the same period of time 50 grams (or approximately the same amount as in Case 4) of hemoglobin nitrogen were manufactured in the presence of a positive nitrogen balance of 16.5 grams. The total cost to the organism in this case was only 32 grams of tissue nitrogen and 2 grams of plasma nitrogen.

The effect of nitrogen retention is even more marked in two other cases (Cases 6 and 9). The demands of nitrogen for hemoglobin manufacture in these patients were met by the nitrogen retained from the food with a small contribution from the plasma. The tissues in these two cases were enabled to lay down nitrogen. *The two cases showing this response differ in one important regard from the other cases. Both patients suffered from extreme nitrogen deprivation.* Indeed, it is doubtful if the tissues could have contributed to an increased nitrogen demand. Experimentally, it was found in Case 6, for example, when the protein intake was much increased, over 1500 grams of protein were laid down in the tissues. In spite of this enormous protein deficit the organism readily spared 46 grams of nitrogen for the manufacture of hemoglobin while the intake of protein was low (Table I, Case 6, Period 1). The requirements of the body for hemoglobin seemed to be greater than those for tissue protein. One fact not shown in the tables must be pointed out in connection with this patient (Case 6). The nitrogen balance did not become positive until after response to anti-pernicious anemia substance was instituted. In short, this patient responded to increased nitrogen demand by still further increasing his nitrogen retention.

The course of events in the second patient (Case 9, Table I), was similar. Here, however, we have unequivocal evidence from the dietary history that tissue starvation had advanced to an alarming degree. Under adequate dietary therapy, without liver or iron, and with a nitrogen

intake of 6.4 grams per day, hemoglobin regeneration occurred. To provide for this additional nitrogen requirement, the nitrogen output in the urine fell to the very low figure of 2 grams a day. For the purpose of giving significance to this statement one may observe for comparison Cases 4 and 5, Table I. These two cases, in which the nitrogen intake was approximately the same, and in which a somewhat faster rate of hemoglobin regeneration occurred than in Case 9, still were able to excrete between 4 and 6 grams of nitrogen daily. Thus hemoglobin demand for nitrogen was provided to a considerable extent from tissue and plasma nitrogen in these cases. In Case 9, however, plasma, hemoglobin and tissue nitrogen were provided by the diet alone in the presence of an exceedingly small nitrogen output.

It will be seen from the foregoing discussion that the body will manufacture hemoglobin under the spur of antianemic substances regardless of the source of the necessary nitrogen. The three responses given above are really different quantitative aspects of the same phenomenon. Which type of response will occur depends upon the level of protein fed, the extent of the hemoglobin demand, and the nutritional state of the organism.

Changes in other factors which have been studied in this series of nine anemic cases will be considered briefly: (1) the albumin and globulin values of the plasma, and (2) the nonprotein nitrogen of the plasma.

*The albumin and globulin values of the plasma.* The albumin content of the plasma was usually below 4.0 grams per 100 ml. On the other hand, the globulin was scarcely ever reduced below normal (1.4 grams per 100 ml.). In only one case (Case 1) was the globulin above 3.0 grams per 100 ml. plasma. Diminution in the total plasma protein during the observations seemed to be usually at the expense of the albumin fraction, the globulin fraction, as a rule, remaining constant. A reduction of the concentration of the plasma proteins could not usually be demonstrated when the need for protein was increased by hemoglobin regeneration, unless the diet contained abnormally small amounts of protein.

*Plasma nonprotein nitrogen.* A striking feature was a very marked reduction of the plasma nonprotein nitrogen in all cases during hemoglobin regeneration. The values reached levels below 20

mgm. per 100 ml. in every case. The lowest recorded values were 14.2 and 14.6 mgm. per 100 ml. in Cases 5 and 8 respectively. The decrease in the nonprotein nitrogen seemed to occur regardless of whether or not there was a decrease of the plasma protein concentration or of the total circulating plasma nitrogen. The values increased again in those cases receiving high protein diets. Increased need for nitrogenous constituents of the circulating plasma during blood regeneration is believed to be responsible for the reduction in the nonprotein nitrogen.

#### COMMENT

It does not seem illogical to assume that in these patients the plasma acted as a vehicle for the transportation and provision of the nitrogen necessary for hemoglobin and cell formation. It may well be that the passage of nitrogen from the tissues to the plasma and so to the hemopoietic centers is considerably greater than the losses of nitrogen from the plasma which are recorded in Table I. The idea of Holman, Mahoney and Whipple (8) from observations in dogs that there is a "give and take" between the tissue proteins and the plasma proteins, seems to have evidence in its favor in these clinical observations.

That there is a tendency to a large conservation of nitrogenous substances in the body, when hemoglobin regeneration takes place, is a fact which has been noted by others (9). Holman, Mahoney and Whipple (10) have demonstrated that there is a large reserve of protein-building substances in the body which can be used to replenish exhausted circulating plasma protein. Presumably, this reserve of protein-building substances can even more readily be employed for hemoglobin formation.

Special comment should be made upon the patient with scurvy (Case 9) who suffered not only from anemia but also from a marked deficiency of nitrogenous substances, as exemplified by the reduced plasma protein, the massive edema, and the markedly positive nitrogen balance throughout the period of observation. It is interesting to speculate upon the conditions which led up to this patient's deficiency in nitrogenous substances. His dietary intake for four years is

quite accurately known, since he partook meticulously during this period of practically only crackers and milk. His diet contained about 1600 calories and about 70 grams of protein or about 11 grams of nitrogen daily. With this intake, he must have had a negative nitrogen balance during at least a good part of the time. Nevertheless, when reparative processes set in, following the administration of a diet adequate in calories and vitamins, it is conceivable that he could have been in nitrogen balance receiving as little as 2 grams of nitrogen daily. It can be inferred from this that vitamin C may play a part in normal tissue protein metabolism, just as it seems to play a part in hemoglobin anabolism.

The practical implication of the results of the present observations is, of course, that in the treatment of anemic patients, attention should be directed to the protein of the diet. Many patients with anemia appear to have a deficient store of nitrogenous substances, and when there is an additional demand on these substances for hemoglobin production the store may be even more depleted. There is no question, however, that there is an additional store of protein substances in the hyperplastic bone marrow of cases of pernicious anemia and chronic iron deficiency. In these conditions there is arrested maturation of nucleated red blood cells, which may fill the marrow spaces of the long bones. The bone marrow, however, can provide probably only a fraction of the necessary nitrogen since in its entirety it is considerably smaller than the volume of the circulating red blood cells (11).

Payne and Peters (12) have presented the idea that a loss of protein into serous effusions in cardiac patients may contribute to edema formation by reduction of the effective osmotic pressure. The concept of an intrinsic loss of body protein by alterations in the internal environment is not new, and is a proposition which must be borne in mind in the management of many types of patients.

#### CONCLUSIONS

1. Hemoglobin formation is accompanied by a positive nitrogen balance, only when the diet contains somewhat more than 6.2 grams nitrogen per day.

2. Hemoglobin formation, however, proceeds at a normal rate even in the face of a negative nitrogen balance.

3. The demand for nitrogen under these circumstances seems to be supplied by tissue and plasma nitrogen.

4. With diets low in protein, extremely low values for daily nitrogen excretion may be observed during blood regeneration, and a reduction of the plasma nonprotein nitrogen is characteristic.

5. The blood plasma seems to play an important rôle in the storage and transportation of nitrogenous substances.

6. In the treatment of anemia a diet adequate in protein is necessary in order to replace tissue nitrogen which may have become diminished before or during hemoglobin regeneration.

#### DESCRIPTION OF CASES

Case 1 (Ly). Female, age 42 years. Hypochromic anemia, chronic loss of blood (menorrhagia), hypochlorhydria, inadequate diet.

Case 2 (Kn). Male, age 42 years. Hypochromic anemia, duodenal ulcer, subacute loss of blood.

Case 3 (Su). Male, age 21 years. Hypochromic anemia, duodenal ulcer, subacute loss of blood.

Case 4 (La). Male, age 30 years. Hypochromic anemia, duodenal ulcer, subacute loss of blood.

Case 5 (Maz). Male, age 41 years. Pernicious anemia.

Case 6 (Mac). Male, age 52 years. Pernicious anemia.

Case 7 (Da). Male, age 82 years. Hypochromic anemia, chronic loss of blood, pulmonary tuberculosis (arrested), diverticulum of esophagus, carcinoma of the stomach (inoperable).

Case 8 (Be). Female, age 72 years. Hypochromic anemia, chronic loss of blood, carcinoma of stomach (inoperable), sensitive carotid sinus.

Case 9 (Ha). Male, age 73 years. Normocytic anemia, scurvy, malnutrition, duodenal ulcer.

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# METABOLIC STUDIES OF THE CHANGES IN BODY ELECTROLYTE AND DISTRIBUTION OF BODY WATER INDUCED EXPERIMENTALLY BY DEFICIT OF EXTRACELLULAR ELECTROLYTE<sup>1</sup>

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The immediate effects of removing extracellular electrolyte from animals without changing the total quantity of body water were described in a recent publication (1). The clinical evidences of dehydration which occurred were shown to be brought about by the shift of water from the extracellular to intracellular fluids.

In the present investigations the depletion of extracellular electrolyte was allowed to persist for seven days. The mechanism of adjustment was studied by frequent blood analyses and determination of the balances of nitrogen, water and electrolyte. Together with more accurate knowledge of the concentration and volumes of intracellular and extracellular water and electrolytes (2), the data are suitable for testing certain hypotheses concerning the factors controlling the distribution and balance of body water and electrolyte.

## EXPERIMENTAL METHODS

Dog 1 (Experiments 3 and 5) was a thin, active male who ate his food well when normal; Dog 2 (Experiments 2 and 4) was a fat female who took the diet less well. Except for the period of deficit of extracellular electrolyte, when food was refused, both dogs were in approximate nitrogen equilibrium or showed a slight positive balance.

The animals were fed the kennel food until a week before the experiments were started, when an artificial diet was given. One kilogram of this diet consisted of commercial casein, 280 grams; sucrose, 250 grams; commercial dextrin, 250 grams; crisco, 200 grams and agar, 20 grams. By analysis, one kilogram contained: nitrogen, 37.8 grams; sodium, 6.4 mM.; chloride, 11.1 mM.; potassium, 2.9 mM. and phosphorus, 71.5 mM.

After a preliminary control period, the dogs were deprived of extracellular electrolyte without significant change in total body water in the following manner. About 100 cubic centimeters per kilogram of body weight of five per cent solution of glucose was injected into the peritoneal cavity. After four hours when considerable amounts of extracellular electrolytes had diffused into the peritoneal cavity, a volume of fluid approximately equal to that injected was removed with a trocar. All food was removed from the cage 18 hours before the peritoneal injection, and feeding was not resumed until the fast had lasted 36 hours. After 7 days, the deficit of extracellular electrolyte was replaced by injecting into the peritoneal cavity a definite amount of a saline solution usually of double physiological strength. The amount of sodium chloride which was injected was greater than that which had previously been removed. In Experiments 2, 3 and 4 the animals were then given only the low salt diet for 7 days and in Experiment 5, for 14 days. In Experiments 3 and 5, subsequently, capsules containing definite amounts of potassium chloride were given daily for 7 days. In Experiment 5, during the last 2 weeks in addition to capsules containing potassium chloride, a total of 350 grams of wheat germ (Embo) were given.<sup>2</sup> The wheat germ taken contained K, 89 mM.; P, 110 mM. and N, 18.6 grams. The preparation did not contain significant amounts of sodium and chloride.

For convenience, the periods will be labeled as follows: Period A, preliminary control; Period B, electrolyte depletion; Period C, administration of sodium chloride; Period D, administration of KCl and Period E, final control.

At the beginning and end of each period and

<sup>1</sup> Read in abstract before the American Society for Clinical Investigation in Atlantic City, N. J., May 6, 1935.

<sup>2</sup> The wheat germ was given as a source of vitamin B, since the diet may be too low in B for prolonged experiments.

at certain other times, analyses of serum and defibrinated blood were carried out.

The methods and calculations of the blood analyses are the same as those used in a previous study (1). Stools and urine were analyzed by the following methods: nitrogen, Kjeldahl (3); chloride, Van Slyke (4); phosphorus, Mackay and Butler (5); sodium, Butler and Tuthill (6) after the removal of potassium as described by Hald (7); potassium, Hald's modification (7) of the method of Shohl and Bennet.

The balance of water was determined by the method of Peters, Kydd and Lavietes (8). The weekly output of calories according to Cowgill (9) would be 4000 for Dog 1 and 5400 for Dog 2. The former figure agrees with the intake for maintenance of Dog 1. The intake for maintenance of Dog 2 was not determined. In calculating the balance of water, the output of calories was assumed to follow the prediction formula of Cowgill and the same figure was used in all periods including the B periods when food was refused. If the caloric outputs were less during these periods, as is likely, the loss of water in these periods would be somewhat greater than those indicated in the table. The water balances are considered to have a possible error of  $\pm 0.075$  liter.

Short protocols for each experiment are appended at the end of the paper.

## RESULTS

As can be seen from the outline of the experimental procedures, the data were collected so as to give information concerning the change in serum electrolyte and the balance of certain constituents of body fluids; (1) when loss of extracellular electrolyte is induced (B periods), (2) when the deficit of extracellular electrolyte is replaced (C periods), and (3) when deficits of nitrogen, phosphorus and potassium are restored (D periods).

### *Symptoms accompanying the procedures*

During the periods of deficit of extracellular electrolyte, the symptoms and signs of dehydration previously described (1) persisted until the loss of sodium chloride was replaced. The animals with large deficits of sodium chloride (Experi-

ments 2, 3 and 4) refused to eat during the entire B periods. Spontaneous recovery of these animals seemed unlikely. Other dogs in similar states vomited if food was in the stomach when the deficit was produced; and in one case after the deficit had persisted 7 days the dog vomited capsules of sodium chloride about 5 minutes after administration. Although salt added to the drinking water would probably have led to recovery, this procedure was not tried.

The oliguria previously noted (1), continued for about 12 to 24 hours. During the period of oliguria little or no water was taken. Thereafter water was drunk in such amounts that water intake and urine output approached that considered characteristic of starvation. In spite of the relatively normal fluid intake, the signs and symptoms of dehydration were unaffected. These observations are mentioned in order to emphasize the following points: (1) dehydration is a phenomenon which does not involve body water alone; (2) diuresis does not develop under certain conditions characterized by a relative excess of water in relation to electrolyte; (3) thirst is not an obligatory accompaniment of dehydration; (4) water intake and urine output may be normal in the presence of dehydration.

In Experiment 5 in which the quantity of extracellular electrolyte removed was considerably less, the symptoms and signs of dehydration were less marked. The dog's activity was but little affected; food was refused for only two days and was taken in normal amounts after 5 days.

Following the restoration of the deficit of extracellular electrolyte by the intraperitoneal injection of a solution of sodium chloride, the signs and symptoms of dehydration rapidly disappeared in all experiments. Food and water were taken in normal amounts, and the dogs behaved in a normal manner throughout the remainder of the experiments.

### *The changes in concentration of serum electrolyte*

Table I gives the concentrations of serum electrolyte per kilogram of water while all other values are expressed in per cent by volume. All samples of blood were withdrawn in the morning which marked the end of the previous 24 hours.

TABLE I

*Concentration of serum electrolyte per kilogram of water*

Experiment number and period	Day	Serum					Cell		
		Water	Protein	Chloride	Sodium	Bicarbonate	Volume	Protein	Water
		per cent	per cent	mM. per liter of H <sub>2</sub> O	mM. per liter of H <sub>2</sub> O	mM. per liter of H <sub>2</sub> O	per cent	per cent	per cent
2A	7	93.3	6.1	116	160	24.3	52.5	36.3	70.7
B	1		8.3		138		65.1	33.6	
	3		8.1		136		57.9	34.9	
	7	92.7	7.0	92	134	24.4	55.7	32.6	67.8
C	3		5.5	111	151		41.1	33.0	
3A	5	93.3	6.8	116	157	23.7	54.5	34.9	71.6
B	1		8.1	91	129		62.8	33.0	
	3		7.7	92	135		60.4	33.0	
	5		7.5	94	135		60.0	34.0	
	7	92.8	7.4	94	140	28.6	58.4	33.6	72.4
C	1		4.8	109	148		36.9	33.8	
	7	93.5	6.1	119	154	21.0	43.0	33.1	72.1
D	7	93.3	6.1	118	155	21.5	48.0	32.2	72.0
E	7	93.5	6.3	115	147	23.5	48.9	33.3	71.8
4A	5	93.0	6.5	116	159	26.0	57.2	35.2	72.0
B	1	91.5	7.8	97	144		68.8	33.6	72.2
	3	91.7	8.2	94	139		62.2	33.5	71.2
	5	92.1	7.8	94	139		60.1	33.1	72.5
	7	92.6	7.1	90	136	27.9	55.0	33.1	72.6
C	1	94.5	5.2	101	137		41.9	33.1	72.8
	3	93.6	5.8	109	154		43.5	33.8	72.2
	7	93.7	5.8	113	155	25.3	41.5	34.2	71.8
D	7	94.0	5.5	117	158	24.1	42.4		
5A	7	93.0	6.9	118	158		55.7	35.1	71.4
B	7	92.5	7.5	110	151		59.3	34.2	72.2
C	7	93.7	6.5	119	149		48.6	35.5	73.7
	14	93.2		120	153		52.3		73.0
D	7	93.0	6.6	115	153		56.1	32.6	73.0
	14		6.5	113	151		57.7	33.5	

The blood findings demonstrate the effects of adjustments taking place during that day.

The immediate changes in the blood produced by loss of extracellular electrolyte are indicated by the samples taken at the end of the first day of Period B; i.e., about 18 hours after the production of the deficit of extracellular electrolyte. The changes to be noted are essentially the same as those described previously for samples taken about 4 hours after the production of the deficit (1). The blood became concentrated as is evidenced by the increased proportion of red cells in whole blood. This concentration resulted chiefly from loss of plasma water as is indicated by the increased concentration of proteins in serum. Increase in erythrocytic water is demonstrated by decrease in concentration of cellular proteins as well as direct determinations of cell water by drying blood and serum. The concentration of serum chloride and sodium was greatly reduced.

The mechanism of these changes was discussed previously (1). Briefly, the alterations are brought about first, by a loss of sodium and

chloride and second, by shift of water from extracellular into intracellular spaces. This transfer of water is necessary to satisfy the disturbed osmotic relationship between intracellular and extracellular fluids which is produced when loss of electrolyte takes place from extracellular fluids without loss of water or loss of intracellular electrolyte.

In Experiments 2, 3 and 4, in which marked deficit of electrolyte was induced, the physiological adjustments during the week following these losses were essentially alike as may be observed from the samples of blood taken during the B periods. As measured by the concentrations of sodium in serum, the osmotic pressure of body fluids remained at low levels throughout the period of deficit. In Experiment 3, a slight increase in concentration of serum sodium occurred but, in Experiments 2 and 4 but little change in serum sodium was found. In all three experiments the concentrations of serum proteins and red cells in blood returned towards normal.

The decrease in concentration of protein in serum and of erythrocytes in blood cannot be interpreted directly as changes brought about by increase in plasma water. Presumably part of the changes are produced by the withdrawal of blood for examination. Exact interpretation is impossible because of the likelihood of destruction or production of these constituents of blood during the periods of observation. The decrease in their concentration which was found on giving sodium chloride would indicate that plasma water remained low throughout the B periods.

In Experiment 5, in which the deficit of extracellular electrolyte was less, the concentration of sodium and chloride in serum returned more nearly to the normal level at the end of the B periods and before the administration of sodium chloride. The increase in concentration of proteins in serum and red cells in blood was about as great at the end of the B periods as it was in the other experiments.

In all experiments, restoration of extracellular electrolyte brought the concentration of serum electrolyte to approximately normal levels. Experiments 3 and 4 indicate that at first the plasma volume was increased beyond normal. During the final periods when potassium chloride was



given, no significant changes in serum electrolyte were found.

### *The balances of nitrogen, water and electrolyte*

The data on the balances of nitrogen, water and electrolytes are summarized in Table II. Although the outputs were determined separately on urines and stools, it was not thought necessary to give these values. The total output may be derived by subtracting the balance from the intake. Since the losses by the stools were small, most of the excretion may be assumed to have occurred by way of the urine. (The stool nitrogen was about 1 gram per week; sodium, 1 to 5 mM.; chloride, less than 1 mM.; phosphorus, 1 to 7 mM. and potassium, 1 to 5 mM.) The interpretations will not depend on the route of excretion.

It is necessary to point out that loss of appetite during the B periods led to absolute starvation in all experiments except number 5. For this reason, the changes in body electrolyte accompanying the deficit of extracellular electrolyte cannot be considered to be pure responses to disturbed osmotic relationships but are complicated by the changes associated with starvation.

During the B periods, the losses of chloride and

sodium were about 25 per cent of the total amounts of these ions in extracellular fluids in Experiments 2, 3 and 4 and about 18 per cent in Experiment 5. These proportions are based on an assumed initial volume of extracellular fluids equal to 27 per cent of the body weights (2).

In Experiments 2, 3 and 4 following the losses of sodium and chloride, a marked loss of body water occurred which was replaced to but a limited extent during the C periods. Nevertheless, the retentions of sodium and chloride in the C periods almost exactly replaced the previous deficits of these ions. Considerable losses of nitrogen, phosphorus and potassium accompanied the losses of water in the B periods. With replacement of extracellular electrolyte during the C periods and return of appetite, the losses of nitrogen and phosphorus ceased, but potassium continued to be excreted during the C periods. When KCl was given in the D periods, large retentions of potassium occurred.

In Experiment 5, but little nitrogen, phosphorus and potassium were lost in the B periods. Nevertheless, a large amount of body water was excreted. During the C periods, extracellular electrolyte was restored without a significant change in body water. Thereafter large amounts of ni-

TABLE II  
*Intake and balance of water and electrolyte*

Experiment number and period	Days	Weight	Urine	Food	Intake						Balance					
					Water	N	P	Na	Cl	K	Water	N	P	Na	Cl	K
		<i>kilos</i>	<i>liters</i>	<i>grams</i>	<i>liters</i>	<i>grams</i>	<i>mM.</i>	<i>mM.</i>	<i>mM.</i>	<i>mM.</i>	<i>liters</i>	<i>grams</i>	<i>mM.</i>	<i>mM.</i>	<i>mM.</i>	<i>mM.</i>
2 A	7	12.7	0.62	402	1.43	15.2	29	3	5	1	+0.04	-6.4	-12	-4	+1	-1
2 B	7	11.6	0.68	0	1.69	0	0	0	0	0	-0.58	-26.5	-60	-94	-77	-50
2 C	5	11.4	1.00	338	2.31	12.8	24	105	107	1	+0.26	+3.3	0	+83	+60	-27
3 A	4	7.1	0.29	480	0.78	18.1	34	3	5	1	+0.05	+1.9	-2	-2	-11	-4
3 B	7	5.8	1.08	0	1.09	0	0	0	0	0	-0.75	-23.7	-54	-71	-60	-57
3 C	7	6.0	0.95	720	1.79	27.2	52	105	108	2	+0.14	-2.7	+5	+88	+64	-27
3 D	7	6.1	0.67	840	1.72	31.7	60	5	54	47	0.0	+9.2	+14	-1	+7	+39
3 E	7	6.05	0.58	519	1.42	20.2	37	3	6	2	-0.06	+0.4	-4	0	-8	-1
4 A	5	12.2	0.36	259	0.77	9.8	19	1	3	1	-0.04	-6.2	-1	-3	-3	-14
4 B	7	11.1	0.91	0	1.61	0	0	0	0	0	-0.66	-30.0	-55	-95	-78	-46
4 C	7	10.5	1.24	308	1.77	11.7	22	128	103	3	-0.03	-3.0	-13	+85	+56	-39
4 D	7	10.1	0.86	152	1.46	5.7	11	1	106	105	+0.23	-6.1	-16	-12	+30	+24
5 A	3	7.4	0.31	405	1.00	15.5	29	3	5	1	-0.06	+3.2	+7	-2	-5	-2
5 B	7	6.5	0.90	260	1.74	9.8	19	2	3	1	-0.57	-5.8	-15	-56	-46	-9
5 C	7	6.8	0.58	840	2.38	31.7	60	5	9	2	+0.12	+13.3	+23	+65	+49	-16
5 C	7	6.6	0.45	560	2.03	21.2	40	4	6	2	-0.11	+6.0	+9	-6	-9	0
5 D	7	6.8	1.40	995	3.88	40.3	114	6	118	152	+0.03	+15.5	+59	-5	+38	+117
5 D	7	6.8	1.93	910	3.94	37.1	108	6	117	151	-0.01	+12.3	+55	0	+6	+62

trogen, phosphorus and potassium were retained without water.

In order to relate these various changes to one another and to the alteration in concentration of serum electrolyte, it will be necessary to mention certain facts about the distribution of body water and electrolyte.

As has been pointed out by Gamble, Ross and Tisdall (10), when concentrations of serum electrolytes do not vary significantly, changes in body water tend to be associated with proportional changes in the other constituents of the body fluids. Thus if the concentration of body electrolyte remains unaltered, a loss of water from extracellular spaces tends to be associated with excretion of the amount of sodium and chloride dissolved in that amount of extracellular water. Similarly, a loss of intracellular water tends to be accompanied by excretion of the amount of nitrogen, phosphorus and potassium present in that amount of intracellular fluid. The same type of relation should also hold for retentions.

Previous work has indicated that the concentration of extracellular sodium and chloride is measured by the concentrations in serum when corrected for content of water and the Gibbs-Donnan effect (11). Analyses of dog tissues and the application of certain calculations (2) permit a reasonable approximation of the volume and concentration of intracellular fluids. Since muscles constitute the largest single reservoir of intracellular water, for certain purposes intracellular water may be represented by the fluid in muscle cells. Our analyses indicate that the concentration of intracellular water of dog's muscles is as follows: nitrogen, 51 grams; potassium, 141 and phosphorus, 104 mM. per liter of water. If balances of potassium, phosphorus and water are assumed to occur in amounts bearing the same relation to nitrogen as was found in muscle cells, the following equations may be used to calculate the expected balances:

$$(1) N \times 0.0198 = \text{balance of intracellular water}$$

$$(2) N \times 2.77 = \text{balance of potassium}$$

$$(3) N \times 2.04 = \text{balance of phosphorus.}$$

N is expressed in grams, water in liters and potassium and phosphorus in millimoles. Table III compares the balances found with those predicted by the above equations.

Experiment 3 may be used to illustrate the reactions in the experiments with the larger deficits (Experiments 2, 3 and 4). As is shown in Table II, the loss of 71 mM. of sodium and 60 mM. of chloride was followed by a diminution of 0.75 liter of body water. Since potassium and phosphorus<sup>3</sup> were also excreted, the change in body

TABLE III

*A comparison of the determined balance of water, phosphorus and potassium with the balances calculated from the balances of nitrogen.*

Experiment number and period	N	Water		Phosphorus		Potassium	
		Found	Calculated	Found	Calculated	Found	Calculated
	<i>grams</i>	<i>liters</i>	<i>liters</i>	<i>mM.</i>	<i>mM.</i>	<i>mM.</i>	<i>mM.</i>
2 B	-27	-0.58	-0.54	-60	-55	-50	-75
2 C	3	0.26	0.06	0	6	-27	8
3 B	-24	-0.75	-0.47	-54	-49	-57	-66
3 C	-3	0.14	-0.06	5	-6	-27	-8
3 D	9	0.0	0.17	14	18	39	25
3 E	0	-0.06	0.00	-4	0	-1	0
4 B	-30	-0.66	-0.59	-55	-61	-46	-83
4 C	-3	-0.03	0.06	-13	-6	-39	-8
4 D	-6	0.23	-0.12	-16	-12	24	-16
5 B	-6	-0.57	-0.12	-15	-12	-9	-16
5 C	13	0.12	0.25	23	27	-16	36
5 C	6	-0.11	0.12	9	12	0	16
5 D	16	0.03	0.31	59	33	117	44
5 D	12	-0.01	0.24	55	24	62	33

water cannot be directly related to losses of sodium and chloride. Table III shows that the negative balance of nitrogen would account for about two-thirds of the actual loss of water and approximately the same losses of potassium and phosphorus as were found. The water excreted in excess of that presumably associated with nitrogen is sufficient to explain the fact that the concentration of serum electrolyte was partially restored during the B periods. For complete restoration with the losses of potassium in Experiment 3, about 0.95 liter of water should have been excreted. Following the administration of sodium chloride, the deficit of extracellular electrolyte was almost exactly replaced and 0.14 liter of water retained in Period C. Since the concentrations of serum electrolyte became normal, the volume of extracellular water must also have returned to the initial value. Hence, the accumulated deficit of water at the end of the C periods must involve chiefly intracellular fluids. Since Table III (B ÷ C) shows that the predicted balances of water, phosphorus and potassium agree essentially with the determined balances of these elements, administration of sodium chloride ap-

<sup>3</sup> In Experiment 4, the balances of Mg and Ca were determined. The losses of these elements were so small that they would not significantly alter the osmotic re-

lationships. In particular the loss of calcium indicates that the losses of phosphorus must be considered to have come from the cells rather than the bones.

parently restored a normal state of distribution of water and electrolyte, except for the changes which were referable to fasting.

Since the diet contained all the elements except potassium which are necessary to rebuild the tissues consumed during the B and C periods, capsules containing definite amounts of potassium chloride were given daily during the D period. This procedure led to retentions of nitrogen, phosphorus and potassium which agree reasonably well with the balances necessary to restore these elements of cellular structures. However, since no retention of water occurred during the period of observation, complete repair had apparently not taken place.

Experiments 2 and 4 show essentially similar results except that a significant excretion of water beyond that accounted for by the losses of nitrogen, potassium and phosphorus and the consequent concentration of serum electrolyte are not demonstrated in the B periods. Although in the D period of Experiment 4, inadequate consumption of food led to loss of nitrogen and phosphorus, a considerable retention of potassium occurred. In this experiment about two and a half times as much potassium chloride was given as in Experiment 3. In this experiment, retention of water accompanied the positive balance of potassium. Apparently continued loss of nitrogen and phosphorus does not preclude the possibility of retaining potassium.

In the B periods of Experiment 5, the loss of 56 mM. of sodium and 46 mM. of chloride was followed by the excretion of 0.57 liter of body water. Since but small losses of nitrogen, potassium and phosphorus occurred, sufficient decrease in body water developed to account for the restoration of the concentrations of serum electrolyte. In the C period, replacement of the deficit of extracellular electrolyte was, however, not accompanied by retention of water. This would indicate that intracellular water was used to restore the volume of extracellular fluids. In view of this finding, it is especially surprising that during the D period a large amount of potassium was retained without water. During the D period almost 100 mM. of potassium were retained in excess of the amount predicted from the balance of nitrogen. However, during the B and C periods, nitrogen and phosphorus had been retained with-

out a corresponding balance of potassium. The excess retention of potassium in the D period is such as to restore the accumulated deficit of this ion with respect to nitrogen and phosphorus.

The lack of retention of water during the C and D periods in this experiment is hard to reconcile with concepts of osmotic equilibrium. During the 56 days of Experiment 5 the balance of nitrogen would lead one to expect a retention of about 0.8 liter of water, but actually a loss of about 0.57 liter occurred. The discrepancy between the determined and predicted balances of water is of the same order of magnitude if calculated from the retention of potassium. In spite of this increase in body potassium without change in body water, osmolar concentration of body water as measured by the concentration of serum sodium remained essentially unaltered. Similar but less striking discrepancies between predicted and determined balances of water may be noted in the other experiments. Table III shows that the divergencies between the predicted and determined balances of water, phosphorus and potassium diminish when Periods B and C are summated. Periods of 7 days may be too short for complete adjustments to take place but it is not certain that longer periods would make all balances of nitrogen, phosphorus, potassium and water occur in constant relations to each other.

#### DISCUSSION

The present data confirm the work of Gamble, Ross and Tisdall (10) and the concepts elaborated in our previous publication and those of Peters and Laviates and others (11, 12, 13). Gamble, Ross and Tisdall found that during the early stage of starvation, loss of potassium occurred in excess of the corresponding loss of nitrogen. During subsequent days, loss of potassium was somewhat less than the corresponding loss of nitrogen so that, at the end of 15 days, the accumulated deficits of nitrogen and potassium had occurred in amounts proportional to their concentration in muscle cells. In these experiments concentration of serum base remained constant and the loss of water could be predicted by the loss of sodium and potassium. In certain of their studies Laviates, D'Esopo and Harrison (13) were able approximately to account for changes in the concentration

of serum electrolyte from the balances of water, sodium and potassium. When similar calculations were carried out on the data in this study, predicted concentrations in serum agreed relatively closely with determined values in some instances but in others widely divergent values were obtained. This discrepancy is most marked in Experiment 5 in which the retention of 179 mM. of potassium without water was associated with no significant changes in concentration of serum electrolyte. Such calculations assume a relatively constant relation between the concentration of sodium in serum and potassium in intracellular water. While osmotic equilibrium between extracellular and intracellular fluid is undoubtedly maintained, variations in the combinations of potassium in the cells may so alter its dissociation as to change the osmotic pressure exerted by a given amount of potassium. Furthermore, the function of the large amount of osmotically inactive sodium (2) in bone and cartilage must be determined before balances of sodium can be assumed to involve only the sodium of extracellular fluids. The present data indicate that the relation of the concentration of serum sodium to that of intracellular potassium must fluctuate rather widely and that loss of nitrogen, phosphorus and potassium can occur independently of each other and of water. Changes in body potassium without change in body water may occur when no significant alteration in concentration of serum electrolyte can be demonstrated. When observations are carried out over longer intervals it may be possible to demonstrate that these substances tend to be retained or lost so as to maintain tissue structures of fairly uniform composition. However, in babies, increased intake of minerals has been shown to lead to increased retentions over long periods (14). Peters and Laviates (12) point out that the relation of body water to tissue solids does not remain constant during periods of changing nutrition and the organism does not give off or take on water in exact mathematical proportions to gains or losses of protein, carbohydrate and fat. Our experiments support these concepts and indicate that balance of body water is not predicted from balances of potassium. However, support is given to the idea that balances of nitrogen, phosphorus and potassium tend to bear fairly constant rela-

tions to each other under the circumstances of our experiments.

The tables show that, when administration of potassium chloride led to retention of potassium, practically all the chloride was excreted. Since by actual determinations as well as theoretical considerations, potassium concentration in extracellular fluids did not increase, the potassium undoubtedly was retained by the cells. These facts support the concept that chloride is probably exclusively extracellular (2).

The present experiments throw light on the physiological adjustments to loss of extracellular electrolyte. In our previous publication the initial response to loss of extracellular electrolyte without water was shown to be maintenance of osmotic equilibrium between extracellular and intracellular fluids by shift of water from the former into the latter. Experiments 2, 3 and 4 show that this state of hydration of intracellular fluids and dehydration of extracellular fluids may persist for a week. The loss of water found in these experiments is practically entirely accounted for by loss of intracellular constituents in the proportions existing in muscle cells. Tissue destruction during starvation explains these losses. In other words the volume of body water was preserved by permitting a decreased concentration of electrolyte to persist.

In Experiment 5, the loss of 55 mM. of sodium led to the excretion of 0.5 liter of water and negligible losses of nitrogen, potassium and phosphorus. Since the concentration of serum sodium was practically restored, the water excreted must have come from the extracellular fluids. The same type of adjustment to moderate losses of extracellular electrolyte, has been demonstrated by Kerpel-Fronius (15), who reported complete restoration of the concentration of serum sodium by excretion of water following the production of deficits of extracellular electrolyte by our technique.

In none of the experiments was potassium not accounted for by loss of nitrogen excreted along with water. Were this to occur, the shift of water into the cells would be avoided, and the volume of intracellular fluid would be protected at the expense of cellular potassium. Loss of potassium without corresponding loss in nitrogen is reported in diabetic coma (16), infantile diarrhea (17),



# PASSIVE TRANSFER ANTIBODIES FOR SIX SAPROPHYTIC FUNGI IN A PATIENT WITH A SUPERFICIAL SCALING DERMATOSIS

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Recent interest in the relationship of fungi to human disease, both as infecting agents and as incitants of phenomena of hypersensitivity, induced us to undertake a detailed study of a patient who presents an uncommon skin lesion which appears to be associated with sensitivity to at least six species of non-pathogenic fungi. Passive transfer antibodies for these fungi were present in the patient's serum, and their reciprocal neutralization by three members of different genera of fungi is of mycologic interest.

## CASE REPORT

The patient, a pathologist of 33, gives no history of hay fever, asthma or other manifestations of hypersensitivity in himself or in his large family, of which he has an unusually detailed knowledge. In September, 1933, he noticed a small follicular papule on the anterior surface of the right mid-thigh. Pus was expressed from this lesion several times, but no distinctive features were noted until late in November, when the lesion, superficial and scaling, involved an area the size of a dime. It was then covered intermittently with adhesive tape, and by December 28 had involved the whole area to which adhesive had been applied.

On January 3, 1934, several similar plaques from 1 to 3 cm. in diameter appeared on the extensor surfaces of the extremities. The evolution of these lesions was very rapid and was associated with intense pruritis.

When first seen on January 12, the original lesion was an irregularly circular, yellowish red, partially denuded and slightly moist area of 4 × 6 cm. There was no tenderness or infiltration and nothing to suggest vesicle or pustule formation. The border was not palpable and showed nothing but adherent scales. The process seemed to be entirely confined to the epidermis, and there was nothing to suggest a follicular distribution. About a dozen similar, but dry, yellowish, and apparently even more superficial, round lesions, varying from 2 to 4 cm. in diameter, were present on the lateral surfaces of the arms, the extensor surfaces of the forearms and the lateral surfaces of the buttocks and thighs. At this time scrapings from the original lesion and from eight of the more recent lesions were inoculated on Sabouraud's glucose agar plates.

One of the eight plates inoculated from the newer lesions became contaminated with a peripheral "spreader"

after several days of observation, and was discarded. No fungus growth appeared on the other seven plates during two weeks of observation, and duplicate cultures from three of these lesions were again negative. The plate inoculated from the original lesion showed numerous colonies of fungi, many of them seeming to arise from the point at which a scale had been deposited. The colonies grew very rapidly but it was possible to isolate seven culturally distinct fungi, all having the appearance of non-pathogenic organisms which have been found on the normal skin (1).

These seven strains have been identified with the assistance of Dr. F. A. Wolfe<sup>1</sup> and Dr. N. F. Conant.

Strain 1. *Cryptococcus*. Group IV (pink), Type A, Benham (2).

Strain 2. *Cladosporium herbarum*.

Strain 3. *Cladosporium* sp. The culture of this strain became contaminated before it could be identified, but examination of a stained smear of the material prepared for skin tests showed fruiting bodies typical of *Cladosporium*. In the gross, the colony was distinctly different from that of *C. herbarum*.

Strain 4. Unidentifiable. This fungus produced a sterile interwoven septate mycelium having no striking characteristics. Sabouraud's glucose agar medium became wine-colored due to the production of pigment. Since no spore forms were produced in culture this strain must be classified with the *Myecelia sterila*. The organism appears to be of a type described by mycologists as an occasional contaminant.

Strain 5. *Penicillium raseo-citreum*.

Strain 6. *Nigrospora* sp. No attempt to determine the species has been made.

Strain 7. *Aspergillus fumigatus*.

The forty-eight hour growth of each fungus was scraped from a Sabouraud's slant and ground in a mortar to break up the larger particles. Sufficient saline was added to produce a slightly cloudy suspension (approximately 0.1 per cent by volume), and the individual suspensions were then sterilized by heating for three hours at 60° C. An intracutaneous test with a mixture of these suspensions produced a large immediate wheal on the patient's forearm, with definite pseudopod formation. There was no delayed local reaction at the point of injection but an increase of the pruritis in all the skin lesions was reported. Intracutaneous tests with the pink *Cryptococcus* produced only an immediate erythema. Each of the other six heat-killed suspensions produced

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an immediate wheal with pseudopods, although these reactions were uniformly smaller than that produced by the mixture. Again, there was no delayed local reaction, but increased itching of the skin lesions was reported.

Blood was drawn for passive transfer tests and then graduated subcutaneous injections of a 1:1000 dilution of the mixture were started. These were followed by such a definite increase in the activity of all the skin lesions that the patient has, until the present time, refused further therapeutic injections and prefers Roentgen ray therapy.

The original lesions healed after this treatment, but one or more superficial, pruritic, scaling areas have recurred at intervals of two or three months, involving different areas on the back of the right hand and on the posterior surfaces of the legs, at, or just above the sock-line. One such outbreak occurred in February, 1935, ten days after the second sample of blood was obtained, and another occurred in January, 1936, about three weeks before the final sample was drawn. The lesions show no tendency to spontaneous resolution but respond readily to Roentgen rays, disappearing without evidence of atrophy or destruction of the hair follicles. There has been no recurrence of the primary lesion, and no lesion has appeared recently on an area completely protected by clothing. No clinical evidence of dermatophytosis has ever been present but a skin test to trichophytin (Metz), 1:100, was positive after forty-eight hours; no immediate wheal appeared.

#### *Passive transfer tests*

##### *Experiment I*

Serum drawn on January 25, 1934, was used within two days of withdrawal to prepare seven sites on the back of each of two subjects (P and M). Forty-eight hours later 0.05 cc. of each suspension of heat-killed organisms, with the exception of the mixed suspension, was injected in each of the prepared sites respectively and in an adjacent control area. No significant reaction to the pink *Cryptococcus* occurred in either subject. Moderate passive transfer reactions (wheals less than 1 cm. in diameter but with definite pseudopods and a marked erythema of 2 to 3 cm.) occurred in each subject to *Cladosporium herbarum* and to *Penicillium roseo-citreum*. Suspensions of the remaining four organisms gave uniformly larger, strongly positive reactions, and the same areas of skin were sensitive to the same amount of antigen forty-eight hours later. All controls were negative. The passive sensitivity was found to persist for at least four weeks.

##### *Experiment II*

On January 24, 1935, another sample of serum was obtained from the patient and a control sample was drawn from another subject who had a somewhat similar skin lesion but did not react to the mixture of heat-killed organisms.

On February 1, twenty-one sites on the back of Subject H. were prepared; the control serum was used in seven, the serum of Experiment I, now 372 days old, in seven, and the fresh serum in the remaining seven. After forty-eight hours 0.05 cc. each of the seven antigen suspensions was injected at sites which had been prepared with each of the three sera respectively and into an area of normal skin. No positive reactions appeared in the untreated areas, or in the sites prepared with the control serum. The old and new samples of the patient's serum, however, appeared to be equally potent in sensitizing this subject's skin. Again, the pink *Cryptococcus* suspension produced no wheal, while the remaining six suspensions produced moderate to marked reactions in the sensitized areas.

The serum of Experiment I was tested a third time after storage for 656 days in the icebox and was found to have lost its sensitizing power.

#### *Neutralization tests*

The demonstration of passive transfer antibodies for six different saprophytic fungi in the serum of an otherwise non-allergic man of thirty-three suggested the possibility that a single reacting substance might be present, particularly when the fungi involved were cultured from a single skin lesion. The neutralization of reagin *in vivo* is now a standard method of testing the identity of atopens, and it has also been shown that *in vitro* neutralization occurs if the reagin is mixed with a suitable amount of the related atopen (3). Experiments were planned to test the unity of the reagin by both methods, in which three of the fungi, *Cladosporium herbarum*, *Nigrospora* sp. and *Aspergillus fumigatus* were used.

##### *Experiment III*

Twelve sites were sensitized by the injection of 0.15 cc. of the patient's serum. Three sites were left untouched, and the remaining nine divided into three groups. The reacting power of each

group to one of the fungus suspensions was exhausted by the repeated injection, at intervals of 7 to 8 hours, of the appropriate antigen in amounts of 0.1 cc. Not more than three injections were needed in any site, and the final testing was done twenty hours after the last injection and forty-eight hours after the sensitizing serum had been given.

TABLE I

Experiment III. *In vivo* neutralization by repeated injection of the sensitized site

Site sensitized on Subject G and:	Tested with suspension of:		
	Cladosporium herbarum	Nigrospora	Aspergillus fumigatus
Exhausted with Cladosporium herbarum	Negative	Negative	Negative
Exhausted with Nigrospora sp.	Negative	Negative	Negative
Exhausted with Aspergillus fumigatus	Negative	Negative	Negative
Controls—sensitized but not treated with antigen	Large wheal pseudopods erythema	Large wheal pseudopods erythema	Large wheal pseudopods erythema

Table I shows the results of the final tests and indicates complete cross-neutralization in all directions.

The experiment is open to the criticism that there was no control of the possibility of non-specific exhaustion of the reacting capacity of the skin, or that the time interval between injections of antigen was too short. In a previous experiment of a similar nature on the same subject the reactions of the test areas were, after an interval of seventy-two hours, identical with those described above, but unfortunately the sensitized control areas could not be found because their marking had worn off. Furthermore, when this subject was used in the preliminary part of Experiment IV, we repeatedly observed large wheal reactions in areas in which a similar wheal had been produced seven to eighteen hours previously. Consequently, none of our results support the idea of an exhaustion of the skin by non-specific means.

#### Experiment IV

In *in vitro* neutralization tests it was found, in a preliminary experiment, that a mixture of two parts of the patient's serum with one part of *Cladosporium herbarum* suspension was capable of sensitizing normal skin but that equal parts of

the two materials resulted in a mixture which did not sensitize. When suspensions of *Nigrospora* and *Aspergillus fumigatus* were used, however, equal parts of serum and suspensions resulted in mixtures capable of producing passive sensitization; two volumes of the antigen were necessary to neutralize the reagin.

Mixtures of a fresh sample of the patient's plasma and each of the suspensions, in proportions appropriate for neutralization, were made and incubated at 37° C. for one hour. Since mixtures of serum or plasma and saline solution which have been treated in this manner produce a non-specific immediate reaction when injected intracutaneously, it was necessary to control the effect of this primary cutaneous reaction on later passive sensitivity. An incubated mixture of equal parts of the patient's plasma and a heat-killed saline suspension of *Rhizopus* was also included as a control. The back of Subject P, who had not been used for 287 days, was prepared on February 5, 1936, and tested sixty-five hours later.

TABLE II

Experiment IV. *In vitro* neutralization of reagin in the patient's plasma by fungus suspensions

Site prepared with 0.1 cc. of mixture	Reaction from mixture	Site tested after 65 hours with	Reaction
Cladosporium + plasma (equal parts)	++++ ++++ ++++	Cladosporium Nigrospora Aspergillus	Negative Negative Negative
Nigrospora (2 parts) + plasma (1 part)	++++ ++++ ++++	Cladosporium Nigrospora Aspergillus	Negative Negative Negative
Aspergillus (2 parts) + plasma (1 part)	++++ ++++ ++++	Cladosporium Nigrospora Aspergillus	Negative Negative Negative
Controls			
Rhizopus + plasma (equal parts)	+++ +++ +++	Cladosporium Nigrospora Aspergillus	++++ ++++ ++++
Saline + plasma (equal parts)	+++ +++ +++ +++	Cladosporium Nigrospora Aspergillus Rhizopus	++++ ++++ ++++ Negative

As shown in Table II, the mixtures of plasma and saline solution and of plasma and suspension of *Rhizopus* produced large wheals which attained maximum size within fifteen minutes; at



these sites normal passive transfer reactions were later demonstrated. The mixtures of plasma and passive transfer antigens, however, produced even larger wheals, which increased in size for about thirty minutes and were still visible as marked erythematous areas after one hour; in these areas no further skin sensitivity could be shown.

The experiment just described was repeated on Subject H, serum instead of plasma being used; the results were the same.

Passive transfer tests, the patient's serum and other antigens than those obtained from him being used, have been done with the following results: *Trichophyton* (Metz) 1:100 dilution, negative in two subjects; *Rhizopus* (from dust), negative in three subjects; *Alternaria* (from dust), positive in two of three subjects; *Monilia albicans* extract (Lederle, 1:100), positive in two subjects; *Saccharomyces cerevisiae*, negative in one subject.

Patch tests to the fungus suspensions have not been carried out on the patient because of the possibility of causing a relatively mild process in the skin to become distressing.

#### DISCUSSION

The dermatosis present in this case resembled a trichophytid seen occasionally in conjunction with severe fungus infections of the feet. No such infection was present, and the character of the primary lesion on the thigh did not suggest an infection with a pathogenic fungus. From a dermatologic standpoint, then, we were dealing with lesions having many of the characteristics of conditions exemplified by trichophytids, which appeared soon after the development of an epidermal reaction around a chronic pustule. It is true that the rapid growth of the saprophytic molds found in this lesion might have inhibited the growth, or prevented the recognition, of types of fungi known to be pathogens. We do not imply that the fungi found produced the original lesion, and have no direct evidence that they were even growing in the skin. However, the rather extraordinary finding of no fungi in seven of the secondary lesions suggested that the isolation of seven fungi from the primary lesion might be of significance. The demonstration of the passive transfer immediate-wheal type of sensitivity to

six of them, the focal reactions following attempts at subcutaneous hyposensitization, and the subsequent recurrence of lesions on relatively exposed areas of skin all lead to the belief that sensitization to saprophytic fungi was an important etiological factor in the manifestations which occurred in our patient following the appearance of the original lesion.

Hilgermann, in 1921 (4), described focal reactions in patients with chronic eczema after injections of vaccines made from cultures of organisms obtained from the lesions, e.g., *Aspergillus*, *Mucor*, *Yeasts* and *Streptothrix*, and numerous types of bacteria. In 1930, Hopkins, Kesten and Benham (5, 6), recorded the case of a man of thirty-seven who gave a history of asthma since childhood and eczema involving exposed areas of skin since the age of twenty-five. Both had cleared up entirely during a sea voyage. "Immediate intradermal reactions" were obtained with extracts of *Aspergillus nidulans* grown from an eczematous area, and of an *Alternaria* and several other fungi grown from dusts. Asthma appeared following the inhalation of spores of these fungi, and "on several occasions following the production of asthma by *Alternaria* spray a patch of eczema became acutely inflamed." Passive transfer was demonstrated for *Alternaria* extract, but no such test with *Aspergillus nidulans* was reported, and patch tests with extracts of the latter were inconclusive. By the routine use of skin tests to extracts of saprophytic fungi in the study of eczema they obtained positive results in twenty-three other patients, five of which improved after a change of environment (7).

Brown (8) reported the cases of two girls of fourteen with eczema occurring on exposed surfaces since infancy, who gave positive cutaneous tests to *Aspergillus fumigatus*; one reacted to tests with *Saccharomyces cerevisiae*. His report and several others (9, 10, 11) indicate that molds are now widely recognized as capable of producing asthma and hay fever in sensitive individuals. Our studies on this case furnish more evidence in favor of the recognition of saprophytic fungi as possible incitants of skin lesions.

Passive transfer reagins are usually present in the serum when the offending antigen produces an immediate wheal in the skin of the sensitive indi-

vidual. Such antibodies have not been demonstrated in cases of contact dermatitis, nor in cases with the delayed, tuberculin type of hypersensitiveness. They are almost constantly present in the hereditary forms of allergy, such as hay fever and asthma. Consequently, the finding of these reagins in a patient with hypersensitiveness should lead to careful consideration of a possible hereditary factor.

It is, however, well known (12, 13) that contact or infestation with *Ascaris* and other worms parasitic on man may produce a lasting skin sensitivity associated with passive transfer substances, irrespective of any hereditary predisposition. Also, Sulzberger and Kerr (14) and Tomlinson (15) have each found one case in which passive transfer reagins for trichophytin persisted for a long time and in which no hereditary factors were present. Each patient had suffered from active dermatophytosis of the feet. Such instances must be very rare, since dermatophytic infection usually produces the delayed, tuberculin type of skin sensitivity; the fungi cannot now be classed with the roundworms as agents which characteristically produce passive transfer reagins. Thus, it seems impossible at the present time to classify the type of hypersensitiveness shown by our patient.

The significance of our failure to demonstrate, by neutralization tests in the skin or in the test tube, more than one substance mediating passive transfer sensitivity to such a variety of fungi as *Cladosporium herbarum*, *Aspergillus fumigatus* and the *Nigrospora* strain is difficult to estimate, since no reports are available which can be interpreted as confirming such a finding. Much evidence supports the attitude expressed by Coca and Grove (3) that, "in the blood of individuals sensitive to more than one substance, more than one reagin can be demonstrated." Consequently, our failure to demonstrate multiple reagins speaks for the biologic identity of some substance common to these suspensions. On the other hand, according to the usual morphologic criteria of mycology, these three genera have nothing in common, except the conidia and aerial hyphae which suffice only to place them in the same large order, "Moniliales," of the Fungi Imperfecti.

It should be noted that we have worked with

suspensions of heat-killed organisms from Sabouraud's agar slants, whereas most other work has been done with powdered felts, with filtered extracts of the fungi or the broth in which they were grown. Our negative results with *Rhizopus* and *Saccharomyces* serve as controls for the medium used, and the simplicity of the methods employed leaves no other obvious source of error.

The group specificity noted in hypersensitiveness to trichophytin (16) suggests a much closer relationship between the dermatophytes than can be demonstrated by morphologic study. Our results indicate that a common substance is present in genera of non-pathogenic fungi which are morphologically unrelated. Perhaps further studies along these lines may be of value in establishing a more satisfactory grouping of the Fungi Imperfecti than present criteria allow. We believe that our findings suggest a closer relationship between the genus *Aspergillus* and the genus *Cladosporium* than is indicated by the present mycologic classification, which places them in separate families on the basis of the "brightness" or "darkness" of the hyphae and conidia.

#### SUMMARY

A man of thirty-three, who gave no history of hypersensitiveness in himself or his family was found to have developed a generalized pruritic scaling dermatosis about five weeks after the appearance of an epidermal reaction around a pustule on the thigh. Culture of the initial lesion, after the dermatosis had appeared, resulted in the isolation of strains of pink *Cryptococcus*, *Cladosporium herbarum* and another *Cladosporium* species, *Penicillium roseo-citreum*, *Aspergillus fumigatus*, a species of *Nigrospora* and an unidentifiable fungus with a sterile mycelium. Plates similarly inoculated from seven other lesions remained sterile. Skin tests with saline suspensions of heat-killed organisms from forty-eight hour growths on Sabouraud's glucose agar slants resulted in immediate reactions to all the strains except the *Cryptococcus*. No late reactions appeared in the test areas, but focal exacerbations of the patient's lesions occurred after both intracutaneous and subcutaneous injections. Cutaneous sensitivity to each of the six fungi was passively transferred to the skin of three normal

individuals. The sensitivity was exhibited by the formation of wheals. By the methods of *in vivo* and *in vitro* reagin neutralization, only a single reagin could be demonstrated for three species: *Nigrospora*, *Cladosporium herbarum* and *Aspergillus fumigatus*. Passive transfer tests were negative to Trichophytin (Metz), *Rhizopus* and *Saccharomyces cerevisiae* but were positive in two of three subjects to *Alternaria* (from dust) and in two subjects to *Monilia albicans* extract (Lederle 1:100).

#### CONCLUSIONS

1. In the patient described there was definite evidence of hypersensitivity to six of the saprophytic molds cultured from the skin. There is reason to believe that this hypersensitivity was an important factor in the development of his dermatosis.

2. Saprophytic fungi should be regarded as possible incitants of allergic dermatoses, as well as of hay fever and asthma.

3. The reciprocal neutralization of reagin by imperfect fungi of three widely separated genera indicates that this test may be of value in the study of the non-pathogenic molds. Other instances of multiple sensitivity to these fungi must be found, however, before definite conclusions can be drawn.

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# CONCERNING THE SPECIFIC RESPONSE OF GUINEA PIG'S RETICULOCYTES TO SUBSTANCES EFFECTIVE IN PERNICIOUS ANEMIA<sup>1</sup>

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## METHODS AND PROCEDURE

Since the value of liver and stomach preparations in the treatment of pernicious anemia was first recognized, attempts have been made to evaluate the potency of the effective materials. Clinical trial, which is the only generally accepted method for testing these products (1, 18), is seriously handicapped by a growing scarcity of patients suitable for assay purposes. Bioassay methods employing laboratory animals have not proven successful. Forty-one such endeavors were listed in a recent review (9). Much interest, therefore, centers about the reports of Jacobson and coworkers (7, 8, 9, 10) that the normal guinea pig may serve as a valid indicator of the therapeutic potency of materials effective in pernicious anemia. Although the use of a normal animal appeared to violate a fundamental physiological principle of replacement therapy, it was deemed worth while to repeat Jacobson's experiments. Having an accumulation of experimental liver extracts awaiting potency determinations, we were eager to confirm the method and apply it to our needs. On the other hand, should the method prove unsatisfactory, an early recognition of this would be desirable. Reports on the potency of various chemically isolated fractions derived from liver and assayed by Jacobson's guinea pig method have already appeared in the literature (3, 20). From experiments based on this method, highly theoretical conclusions are also being drawn concerning the relation of congo red to the pathological physiology of pernicious anemia (13), and the reaction of normoblastic bone marrow to liver extract (11). Finally, the danger inherent in the use of an unreliable bioassay method by pharmaceutical firms is obvious.

The method of Jacobson, as outlined in his latest publication (9), was followed precisely in the great majority of our experiments; all deviations will be specifically noted. In order that the following experiments may be clearly understood sufficient details of Jacobson's technique will be given.

*Selection and care of animals.* One hundred and eighteen normal adult guinea pigs weighing from 300 to 800 grams were used. About one-third of the animals were obtained from several local sources, and the remainder were received from reputable breeders in other cities. For convenience, we chose to work chiefly with male guinea pigs, as did Jacobson; only 20 were females. Wire cages with large-mesh screen bottoms housed from 4 to 8 tenants each. The diet consisted of oats, carrots and lettuce, as Jacobson recommended. In earlier experiments on 44 guinea pigs, cabbage was used in place of lettuce. A small number of animals, in an experiment to be described later, was placed on a diet producing black tongue in dogs.

*Reticulocyte counting.* In some preliminary experiments the "permanent preparation" method of Osgood and Wilhelm (17) was employed. This required about 0.05 cc. of blood obtained from a minute ear vessel; thin and well-stained preparations were easily made. In the great majority of experiments, however, the simpler "wet preparation" technique employed by Jacobson was followed.

Since we regarded a count of only 500 red blood cells, as employed by Jacobson, to be uncertain in accuracy for low reticulocyte ranges, our reticulocyte estimations were based on no less than 1000, and frequently 5000 to 7000 cells. The counts were made by individuals experienced in the technique. Each preparation was treated as an "unknown," and it was a frequent practice for the observers to exchange slides for mutual checking. The reliability and uniformity of the results were thus insured.

*Method of administering liver extracts.* All solutions, with either water or normal saline as the diluent, were made up to a volume of 5 cc. and given intraperitoneally in a single dose. Lederle's Solution Liver Extract Parenteral, N.N.R., was used. Jacobson's basic dose was adopted, namely, the extract derived from 4.3 grams of fresh liver per kgm. of guinea pig. In most experiments,

<sup>1</sup> Aided in part by a grant from the Committee on Scientific Research of the American Medical Association.

however, 10, 100 and 240 times this amount was injected.

*Control injections.* In addition to potent liver extracts, two control materials were employed: sterile physiological saline solution and inactivated liver extract. Inactivation was accomplished by heating the liver solution to above 200° C. for 30 minutes, and boiling the resulting charred material for 4 hours. A third control procedure consisted in daily reticulocyte counts on uninjected animals for extended periods.

*Selection of reactive guinea pigs.* The assay method of Jacobson is based on the use of so-called "reactive" guinea pigs; these animals are said to exhibit a specific reticulocyte response to liver extract. The criteria for their selection, and the fundamental characteristics of their reticulocyte behavior, as described by Jacobson, may be briefly summarized as follows:

1. If the reticulocyte values of an unselected group of newly acquired adult normal guinea pigs are followed for an initial test period of 8 days, it is stated that the large majority will consistently show levels below 1.2 per cent. Animals with reticulocyte percentages higher than 1.2, or those which do not fall to this level or lower and remain there for the last 4 days of this preliminary test period, are discarded as "unstable."

2. From 30 to 70 per cent of these stable animals are said to exhibit a reticulocyte response to potent liver extract. These guinea pigs comprise the group known as "reactive." This means that at some time within the 6 days following the injection, the reticulocytes should rise to 2.0 per cent or over for 2 consecutive days. Only animals that are reactive in this manner to the initial test dose of liver are retained for further use as assay subjects.

3. Guinea pigs once reactive are said to be permanently reactive to the injection of potent liver extracts, and by virtue of this remain suitable indefinitely for assay purposes.

*Hematopoietic response to liver extracts.* Inasmuch as stable and reactive guinea pigs, as defined above, are said to remain stable and to maintain indefinitely their specific reticulocytic responsiveness to liver with an approximately constant degree of sensitivity, Jacobson uses the reactive animals in the assay of unknown liver preparations. Furthermore, quantitative determination of potency is believed possible, since it appears that there is a threshold value for the response; and Jacobson places this minimally effective dose of potent liver at the amount of extract derived from 0.6 mgm. of fresh porcine liver per kgm. of guinea pig. This amount is termed a guinea pig unit (G.P.U.). When a reactive guinea pig is given one G.P.U. or any multiple of this, the reticulocytes will rise within 6 days to 2.0 per cent or over for 2 consecutive days. It is stated that this response is all or none, and bears no relation to the amount of liver injected above the minimally effective dose; nor is there any other recognizable hematopoietic change of significance. In the assay of an unknown product, Jacobson accepts a positive response in 2 out of 3 injected test animals as evidence of the presence of the anti-pernicious anemia substance. By testing various known dilutions, the potency of the

material can be quantitated. The reactive animals, after a suitable rest period, are used over and over again; one animal was used 18 times in 21 months by the author of the method.

*Fundamental postulates of the method.* It is obvious that in order for the guinea pig assay method to be valid, the two following fundamental criteria must be fulfilled unequivocally:

1. A "stable" animal must remain stable.
2. A "reactive" animal must show a reticulocyte response only to potent anti-anemia preparations, and not to indifferent substances; and it must remain reactive.

If either or both of these postulates cannot be substantiated, the method in our opinion is unsatisfactory.

## RESULTS

To test the two basic tenets set forth above, 1977 reticulocyte counts were performed in 260 experiments on 118 guinea pigs.

*Selection of "stable" guinea pigs.* Our results show an extreme variability in reticulocyte levels of normal adult guinea pigs when observed for an eight-day period. This variability was observed not only in individual animals, but also in groups of guinea pigs. For example, in one group of 12 guinea pigs, 10 were stable on preliminary testing, but in another batch of 24 animals, only 1 was found stable.

When all our results on preliminary stability tests were analyzed nearly 48 per cent of all guinea pigs had reticulocyte levels which rendered them unsuitable in the sense described above. This figure is greatly in excess of that reported by Jacobson (9), who claimed that the large majority of all animals were stable on first trial. Regardless of this discrepancy, the question immediately arose whether the stable group, representing 52 per cent of our guinea pigs, would remain stable. We tried to answer this question in two ways. First, liver injection was withheld from a group of stable animals, and their reticulocytes were followed daily for from 8 to 20 days. Secondly, instead of discarding those found unstable on first trial, a large group was followed through second and third observation periods. In this manner it has been possible to learn much concerning the reticulocyte fluctuations occurring spontaneously in normal guinea pigs.

In regard to the so-called stable group, 25 animals which met the requirements on first trial were allowed to rest for one or more weeks, and they were then re-examined for the stability of their reticulocyte levels. Thirteen were found to have more than 2.0 per cent reticulocytes for 2 consecutive days. This is at variance with the results of Jacobson (9), who reported that his stable group did not exceed 1.6 per cent reticulocytes when retested. If these animals had been injected with liver extract, these spontaneous reticulocyte rises might have led us to believe that

over 50 per cent of the guinea pigs had responded positively. Typical charts of these experiments are shown in Figures 1 and 2, which illustrate a mild and an extreme instance, respectively, of spontaneous fluctuations of reticulocytes in previously stable animals.

It is obvious from these experiments that guinea pigs show spontaneous reticulocyte fluctuations of such a character that they cannot be depended upon to remain stable simply because they had had low reticulocyte counts during *one* trial period. Indeed, our results indicate that slightly

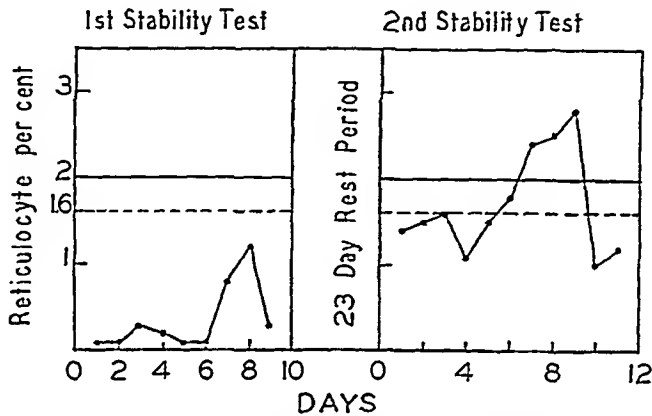


FIG. 1. GUINEA PIG NUMBER 17, ♂, 580 GRAMS. MILD SPONTANEOUS RETICULOCYTE FLUCTUATION IN A PREVIOUSLY STABLE ANIMAL.

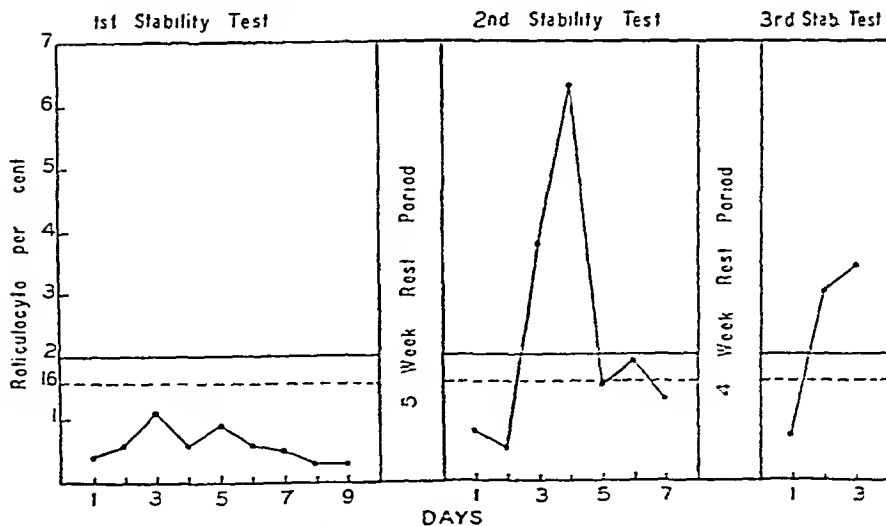


FIG. 2. GUINEA PIG NUMBER 25, ♂, 520 GRAMS. EXTREME SPONTANEOUS RETICULOCYTE FLUCTUATION IN A PREVIOUSLY STABLE ANIMAL.

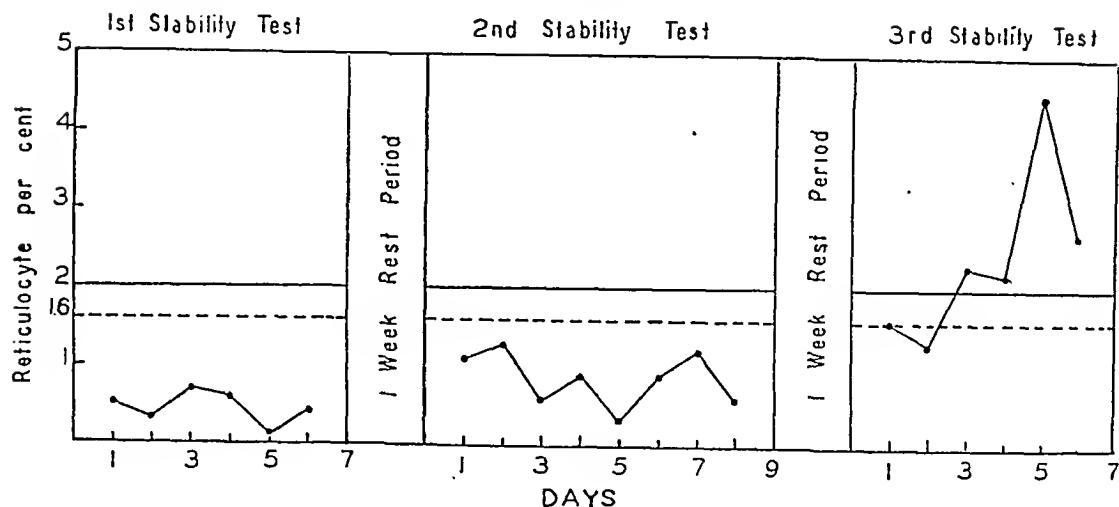


FIG. 3. GUINEA PIG NUMBER 50, ♂, 740 GRAMS. SPONTANEOUS RETICULOCYTE FLUCTUATION AFTER TWO PREVIOUS STABLE PERIODS.

over 50 per cent of all guinea pigs stable during one observation period will be found unstable if re-examined at a later time. Furthermore, if one now takes those animals remaining stable through a second trial period and tests them a third time, again an appreciable number will be found unstable. For example, in one group of 11 guinea pigs stable during two previous observation periods, 5 were no longer stable when subjected to a third trial. In Figure 3, this occurrence is illustrated, and the experiment depicted is typical of 4 others.

From these experiments it is clear that in the guinea pig reticulocyte values fluctuate spontaneously. The reticulocyte behavior during any *single* period of observation, therefore, is not a valid basis upon which to label an animal stable or unstable. The particular result obtained depends entirely upon what part of the reticulocyte curve is being observed. If a stable guinea pig is subjected to repeated periods of observation, it will sooner or later be found unstable. This is well seen in Figure 4, which illustrates continuous daily reticulocyte counts taken on a stable animal over

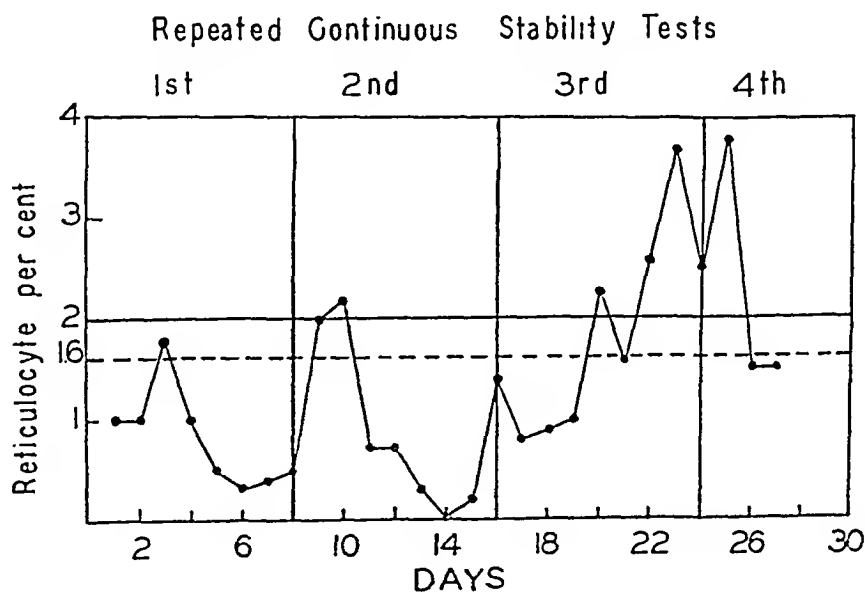


FIG. 4. GUINEA PIG NUMBER 14, ♂, 540 GRAMS. SPONTANEOUS RETICULOCYTE FLUCTUATIONS DURING PROLONGED OBSERVATION OF A PREVIOUSLY STABLE ANIMAL.

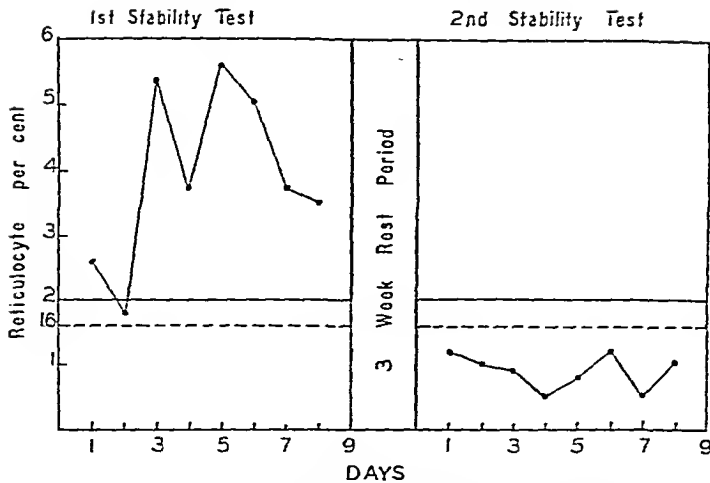


FIG. 5. GUINEA PIG NUMBER 98, ♂, 510 GRAMS. STABLE RETICULOCYTE PERIOD IN A PREVIOUSLY UNSTABLE ANIMAL.

a period of 20 days, and is typical of 5 similar experiments.

Next, a study was made of the reticulocyte behavior of those animals which were unstable in the *first* observation period. Instead of being discarded, as Jacobson recommended, these animals were allowed to rest and were then re-examined. Of 29 such animals, 14 or slightly less than 50 per cent were found to be stable when given a second trial. Of the remaining 15, which were again unstable during the second trial period, 10 were allowed to rest and then tested a third time. Of these, 6 animals were now found stable. In other words, only 4 animals of the original group of 24 that were followed remained unstable through three trials. It is probable that if these 4 animals were subsequently retested

they too would ultimately have stable reticulocyte periods. The importance of these experiments lies in the possibility that if by chance all initially unstable guinea pigs had been observed a few weeks earlier or later, a large number would have been accepted as reliable test animals instead of having been discarded. Figure 5 shows an experiment on an unstable guinea pig, which, when tested three weeks later, was found stable; this experiment is typical of 13 similar ones. Figure 6 illustrates that an animal, unstable during two separate trial periods, was found stable on the third testing; there were 5 experiments with similar results.

From the above evidence, it may now be said that not only is it impossible to rely on the reticulocyte constancy of a so-called stable animal, but

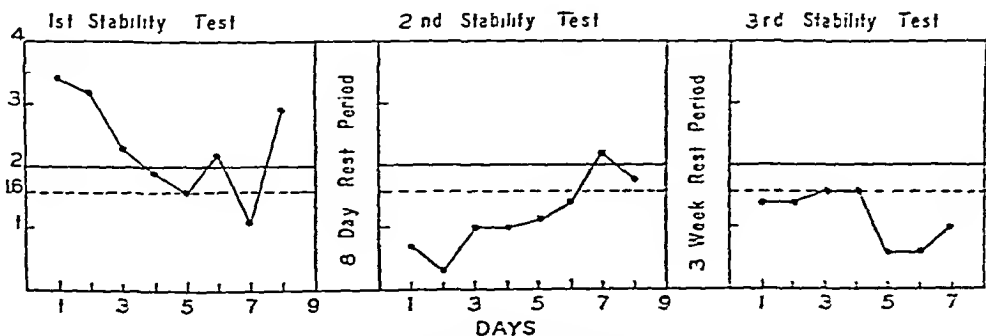


FIG. 6. GUINEA PIG NUMBER 105, ♂, 480 GRAMS. STABLE RETICULOCYTE PERIOD AFTER TWO PREVIOUS UNSTABLE PERIODS.



it is also impossible to discard a particular guinea pig as unstable. The results, in our opinion, definitely indicate that if one is guided by the criteria of the method, all guinea pigs are unsuitable because of their normal, spontaneous and unpredictable reticulocyte fluctuations. It has not been possible, therefore, to substantiate the first postulate of the method.

*The specificity of the reticulocyte response to liver injections*

*I. Response to injection of liver.* Despite the marked and unpredictable fluctuations of reticulocytes occurring in normal guinea pigs, we deemed it advisable to investigate the method further to discover whether there might be some characteristic response to potent liver easily distinguishable from any *spontaneous* change in reticulocyte level.

After a short rest period, potent liver extract was administered to 44 guinea pigs meeting the qualifications for stability. The experiments were divided roughly into four groups given respectively Jacobson's routine dose and also 10, 100, and 240 times this dose. Positive responses were obtained in 15 experiments, or 34 per cent. No difference was noted in regard to the amount of liver injected. The group receiving the smallest dose showed the same variability of reticulocyte behavior as the group receiving the largest dose. In Figure 7 is illustrated a positive and negative experiment in the *same* animal which on two occasions, separated by a suitable rest period,

received 240 times the routine dose of liver extract. A positive reticulocyte response appeared to follow the first injection; there was no apparent effect from the second injection. A similar result was obtained in 2 other experiments.

These experiments indicate that a guinea pig which has responded positively cannot be relied upon thereafter always to respond positively to liver. Jacobson's contention, therefore, that "the reactive state is maintained indefinitely" was not confirmed.

*II. Response to injections of control substances.* Observations made on normal uninjected guinea pigs constitute important control data. Additional control experiments were performed by injecting isotonic solutions of sodium chloride and totally inactivated liver extract. The latter was given in doses corresponding to those used for the potent extracts. The method of inactivation has been described.

Injections of salt solution were made in 27 stable guinea pigs. Many of these animals had undergone prior injections of liver or repeated stability observations; the remainder received the saline injection directly following the first stability test. Ten of these 27 experiments resulted positively, and thus 37 per cent of the nonspecific saline injections produced the type of response reserved by the Jacobson method exclusively for potent liver materials.

Injections of inactivated liver were made in 16 stable guinea pigs, and 10 of these, or 63 per cent,

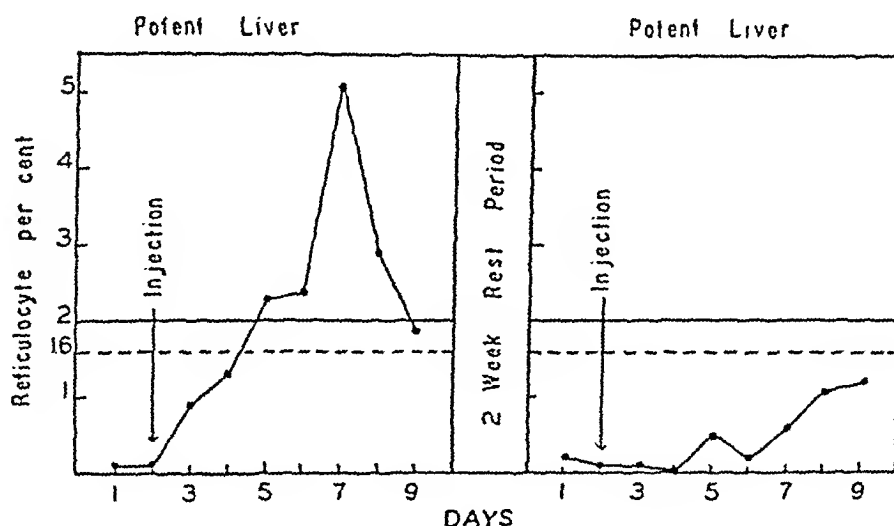


FIG. 7. GUINEA PIG NUMBER 18, ♂, 445 GRAMS. NEGATIVE RESPONSE TO LIVER INJECTION IN A PREVIOUSLY REACTIVE ANIMAL.

were positive. In other words, the majority of the animals reacted positively to impotent liver.

From these control results, it appears that here again one is observing only normal spontaneous fluctuations of reticulocytes, and that the injections of liver extract or saline are irrelevant and merely coincidental.

In Figure 8 is shown an example of a positive response to the injection of saline solution.

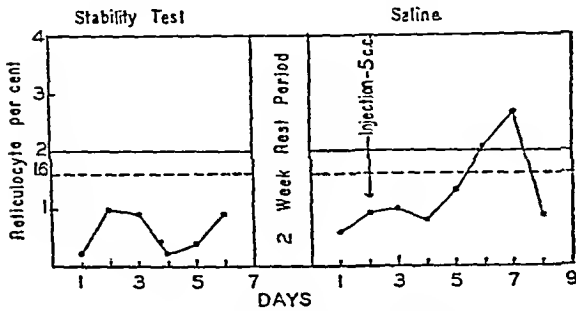


FIG. 8. GUINEA PIG NUMBER 54, ♂, 535 GRAMS. POSITIVE RESPONSE TO SALINE INJECTION.

Figure 9 illustrates a positive response to the injection of inactivated liver extract solution.

In Figure 10 is depicted a control experiment in a stable animal which had reacted positively to the injection of liver. Then, to test the specificity of the liver response, saline and inactivated liver injections were given. Positive reticulocyte responses followed each of the control injections. This experiment is typical of 5 others.

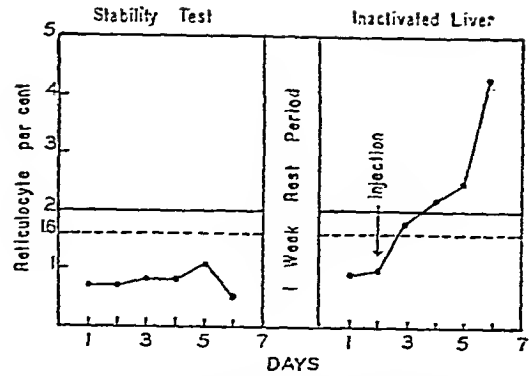


FIG. 9. GUINEA PIG NUMBER 48, ♂, 625 GRAMS. POSITIVE RESPONSE TO INACTIVATED LIVER INJECTION.

A comparison was made of the reticulocyte curves of *all* positive responses including those occurring spontaneously and after active liver, inactivated liver, and saline injections. The analysis included the character of the curves in regard to contour, the average height of reticulocyte peaks, the total reticulocyte response, the duration of the response, and the day on which the peak was reached. No features were found which would distinguish the potent liver responses from the non-specific ones. The results are listed in Table I.

From the evidence presented in this section, therefore, it is clear that the second postulate concerning the specificity and permanency of the reticulocyte response to liver extract could not be substantiated.

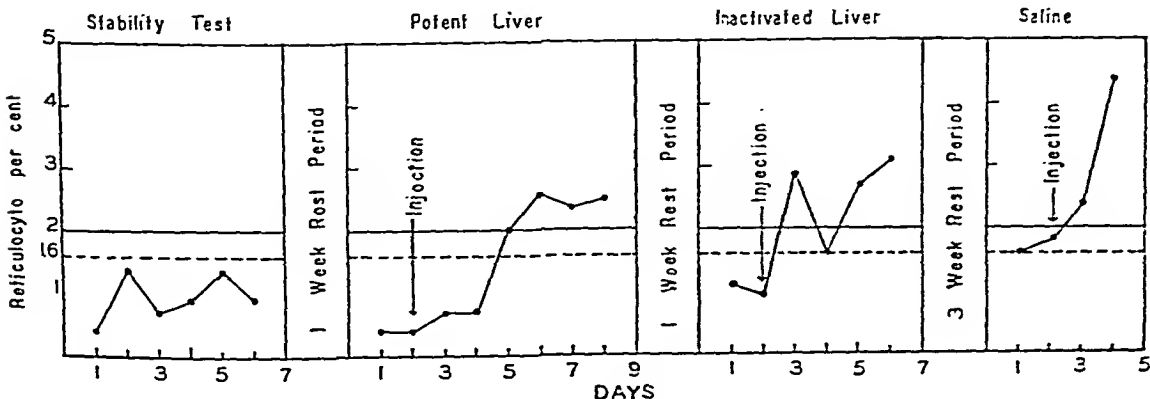


FIG. 10. GUINEA PIG NUMBER 46, ♂, 580 GRAMS. POSITIVE RESPONSES TO INACTIVATED LIVER AND SALINE IN A PREVIOUSLY STABLE AND REACTIVE ANIMAL.

TABLE I \*  
Analysis of positive reticulocyte responses  
in stable guinea pigs

Treatment	Reticu- lyocyte peak	Dura- tion of re- sponse	Day of peak response	Total reticu- lyocyte response figure †
	per cent	days		per cent
Active liver (15 ani- mals).....	3.9	4	4th	2.9
Saline (10 animals)....	3.5	4	4th to 5th	2.8
Inactive liver (10 ani- mals).....	3.2	3	4th	2.6
Uninjected (13 animals)	3.8	4	3rd to 4th	3.2

\* All figures represent the average of the values for all experiments in each group.

† This figure is obtained by averaging those reticulocyte values over 1.8 per cent during the period of response.

The method, we believe, has established by definition an *arbitrary* division between stable and unstable guinea pigs, and positive and negative responses. It is our experience that this dividing line of 1.2 per cent reticulocytes, which differentiates between stable and unstable animals, is drawn near the mean in the range of reticulocyte fluctuations of normal guinea pigs. For example, if our interpretation is correct concerning the *spontaneous* character of these reticulocyte values, it would be of interest to know the range of distribution of the reticulocyte counts obtained in all our experiments. This is shown in Figure 11, in which curve A represents the distribution of 1977 reticulocyte counts done on 118 guinea pigs regardless of the various procedures followed in particular animals. It is interesting that 1029 counts, or 52 per cent of the total, are 1.2 per cent or below. This correlates well with the previous finding that approximately one-half of our guinea pigs were stable.

In Figure 11, curve B represents the distribution of the reticulocyte counts of *all* the potent liver experiments; curve C, similarly, represents the distribution of the reticulocyte counts of *all* the control experiments. There is no significant difference in the character of these reticulocyte distributions.

It is merely chance that in any fairly large group of animals, regardless of various harmless procedures, a certain number will show reticulocyte values over 2 per cent for 2 consecutive days. The definition of a positive response is, in our opinion, an arbitrary one.

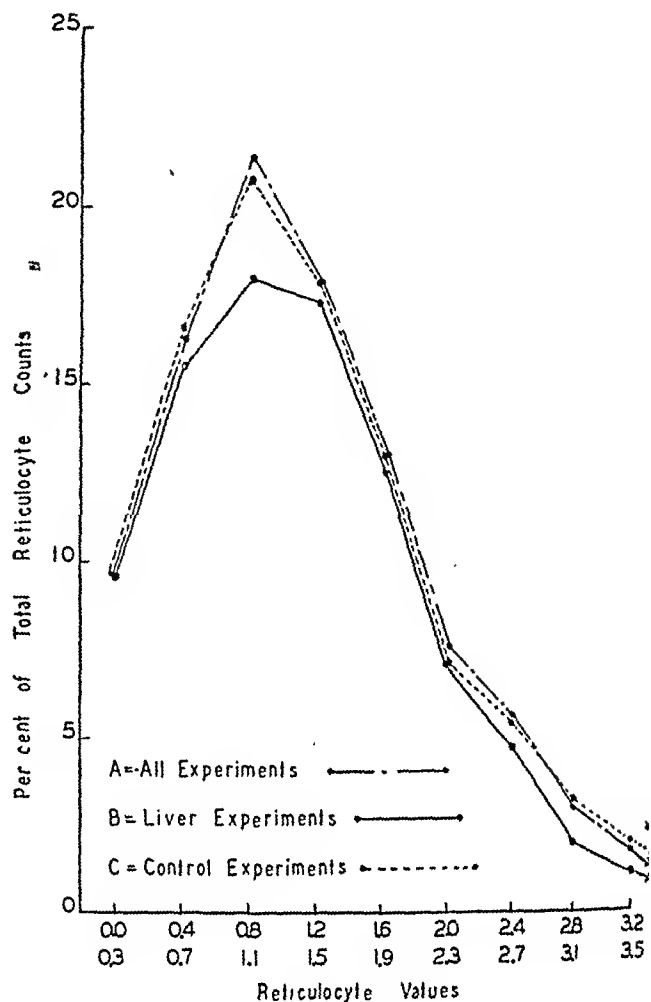


FIG. 11. DISTRIBUTION OF 1977 RETICULOCYTE COUNTS REPRESENTING ALL EXPERIMENTS (CURVE A), AND COMPARISON WITH 439 COUNTS IN LIVER EXPERIMENTS (CURVE B), AND 1538 COUNTS IN CONTROL EXPERIMENTS (CURVE C).

In Table II is presented a statistical summary of all the experiments performed on stable guinea pigs.

TABLE II  
Statistical summary of results of experiments performed  
on stable guinea pigs

Treatment	Number of experi- ments	Positive responses		Negative responses	
		Num- ber	Per cent	Num- ber	Per cent
Active liver.....	44	15	34	29	66
Saline.....	27	10	37	17	63
Inactive liver.....	16	10	63	6	37
Uninjected.....	25	13	52	12	48

Results in regard to sex, source and diet. No differences were noted in groups of guinea pigs acquired from different sources. The data presented by males and females were identical. The

results in the preliminary experiments with 44 guinea pigs given cabbage as the green vegetable were the same as the results with the 74 guinea pigs fed lettuce; nor were there any changes when the cabbage-fed animals were later placed on lettuce.

*Hematological observations.* In 23 animals the main features of the blood picture were studied.

All counts were done in duplicate, using blood pipettes and counting chambers. WBC readings were made on the Haden-moglobinometer calibrated by the oxyt determination. Packed red cell volume measured with Van Allen hematocrits, treated with 10 per cent potassium oxalate solution. Cyte extreme findings are presented in Table III.

TABLE III  
any increase on 23 normal guinea pigs

the blood picture	Hemoglobin	Hematocrit
variable reticulocyte count	grams per cent	per cent of packed cells
resent only the u.s.	14.7	42.0
pig's marrow. It is	16.0-13.0	47.0-37.0
in the absence of a		

efficiency, no characteristic injection of liver extract

*Results in regard to method.* When a completely different data obtained by the "old" method of Osgood and We used in about 20 per cent of the "fresh" preparations and the remainder, no significant

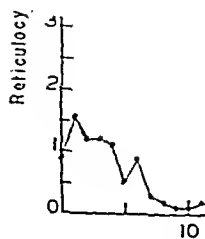
and fall of these correlated with and detectable in suggests that the these animals represent the normal guinea

remarkable, then, that specific hematopoietic deficit response follows the

to reticulocyte counting comparison was made of the permanent preparation"

Wilhelm (17), which was not of our experiments,

FIG. 14. GUINEA PIG N° 11 "method used in the differences were noted.



The small daily loss of blood incidental to the reticulocyte estimations had no effect on the reticulocyte values or blood count. This was determined in 6 animals that were bled daily far in excess of the amount of blood required for the reticulocyte counts.

In our opinion a reticulocyte percentage based on a count of only 500 red blood cells is unreliable in these low reticulocyte ranges. Unusual care was exercised to insure perfection in the preparation of the blood films; nevertheless, the values obtained for duplicate reticulocyte counts on blocks of 500 cells each were often significantly different. The necessity for counting at least two blocks of not less than 500 cells each is seen in Figure 12 which illustrates a few of the more striking instances in which, by counting only 500 cells, one might conclude that the animal was stable or non-reactive, and then by counting a different block of 500 cells the animal would be judged unstable or reactive.

We do not feel that the presentation of these data labels our counting technique any the less accurate, but rather that it indicates a source of difficulty and possible error in a relatively simple hematological procedure even when done carefully by experienced workers. It is our understanding that the accepted standard for reticulocyte counting in clinical work is a minimum of 1000 red blood cells for reticulocyte levels under 5 per cent. Since Jacobson's method necessitates the differentiation between 1.5 and 2.0 per cent of reticulocytes, permitting an error of less than 3 reticulocytes per 500 red cells, it would seem even more necessary to examine at least 1000 cells.

*Reticulocyte behavior of guinea pigs on the Goldberger diet producing black tongue in dogs.* Inasmuch as we were unable to obtain truly stable guinea pigs, it was thought desirable to attempt to stabilize the circulating reticulocytes at a low level and to render the bone marrow specifically susceptible to liver extract. We sought to accomplish this by feeding a deficient diet. The results of some of these experiments (2) are reported here because they shed additional light on the bone marrow activity of this species. The many lines of evidence which suggested the use of the diet producing pellagra can be indicated only briefly here. In Goldberger's hands the diet

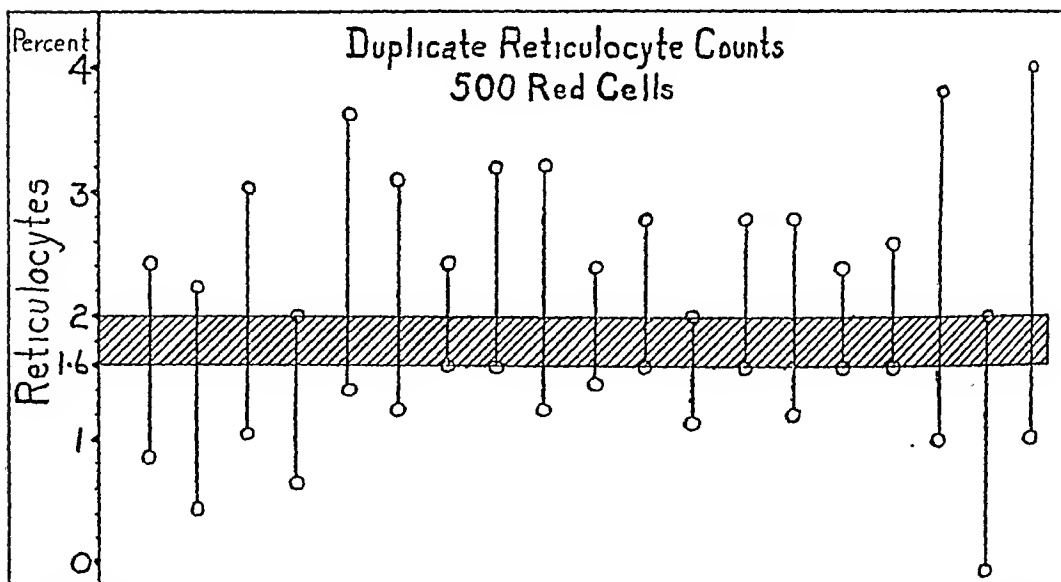


FIG. 12. 19 REPRESENTATIVE INSTANCES OF SIGNIFICANT DIFFERENCES IN DUPLICATE RETICULOCYTE COUNTS, OF 500 CELLS EACH, MADE ON THE SAME PREPARATION.

Note that in each instance the duplicate counts fall on opposite sides of the shaded "crucial zone" which defines reactivity and the upper limit of stability.

was productive of pellagra in humans (5). Rhoads and Miller (19) reported that this diet caused anemia and black tongue in dogs, while in swine it produced a syndrome strikingly similar to human pernicious anemia (15). The diet has been thought to be deficient in vitamin B<sub>2</sub>, and the relationship between the anti-pernicious anemia principle and vitamin B<sub>2</sub> has received considerable attention. Finally, Miller and Rhoads found that the anti-anemia substances, when fed orally, possessed a life-saving factor for guinea pigs maintained on this diet (14).

Guinea pigs were fed the diet producing pellagra (4, Table 6) plus orange juice, but omitting sucrose, and their blood pictures were followed. In the majority of these animals the reticulocytes decreased within two weeks to extremely low levels. In many instances no reticulocytes were seen even on counting as many as 5000 to 10,000 red blood cells, and counts of 0.02 per cent were not rare. In 4 animals showing such extremely low counts for from several days to a week, single intraperitoneal injections of 0.25 cc. of Lederle's concentrated liver extract solution were given. The reticulocytes rose rapidly in 3 guinea pigs, more slowly in the fourth one, but in all instances there occurred what appeared to be a definite and marked response with an increase of reticulocytes to from 2.0 to 2.5 per cent. As a control, there-

fore, another group of 4 animals on the same diet was observed daily, and it was noted that there occurred spontaneous increases in reticulated cells much greater than those observed after liver extract. In 2 animals that lived on this diet for two months, several periods of alternating extremely low and very high reticulocyte levels were seen, and these phasic variations were related neither to any observable change in the well-being

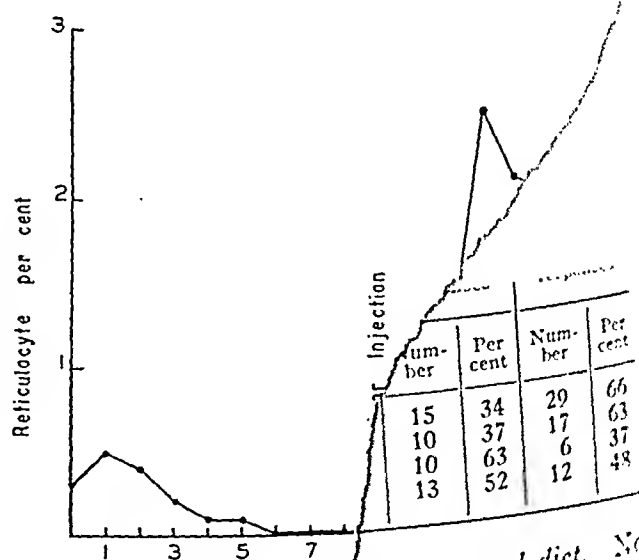


FIG. 13. GUINEA PIG 14 groups of guinea pigs GOLDBERGER DIET. APPARATUS. The data pre- LIVER INJECTION. sources were identical. The

of the guinea pigs nor to the loss of weight which occurs on this regimen. Here again, then, one finds marked fluctuations in animals which for short periods of observation appear to have reduced bone marrow activity and low and stable reticulocyte values induced by a deficient diet. Figure 13 illustrates an apparent effect of liver injection given during a period of reduced bone marrow activity, and Figure 14 presents a comparable experiment in which liver injection was withheld but the reticulocytes counted daily for over two months.

From these experiments it seems that this diet altered bone marrow activity in such a manner that the swings in peripheral reticulocyte levels seen in normal guinea pigs were markedly accentuated. The reticulocyte behavior on the deficient diet differed not in kind but only in degree from that of normal animals. Thus, what appeared to be a promising method for rendering the guinea pig a suitable assay animal has failed in our hands.

It should be added that the daily oral administration of large amounts of Lilly's Liver Extract, N.N.R., to these guinea pigs had no characteristic effect on the reticulocytes. The response obtained by Jacobson (9) from the oral admin-

istration of anti-anemia substances to normal guinea pigs finds no support in these experiments.

#### DISCUSSION

The failure of our experiments to corroborate Jacobson's assay method is clear from the results presented. This discrepancy cannot reasonably be ascribed to an inherent hematological difference between his animals and ours. We used normal adult guinea pigs deliberately obtained from several sources. The animals remained healthy, and the blood pictures in a representative group were entirely normal throughout the experiments.

Jacobson (9) suggested that the megaloblastic bone marrow which he observed in his reactive animals bore a close causal relation to their suitability for assay purposes, and stated that "the large number of megaloblasts in proportion to the number of normoblasts, simulate the classical picture of the bone marrow findings in pernicious anemia." He found, however, the same type of marrow in the non-reactive guinea pigs, and from the remainder of the hematological data it is probable that his finding is the normal condition for this species. A megaloblastic marrow oc-

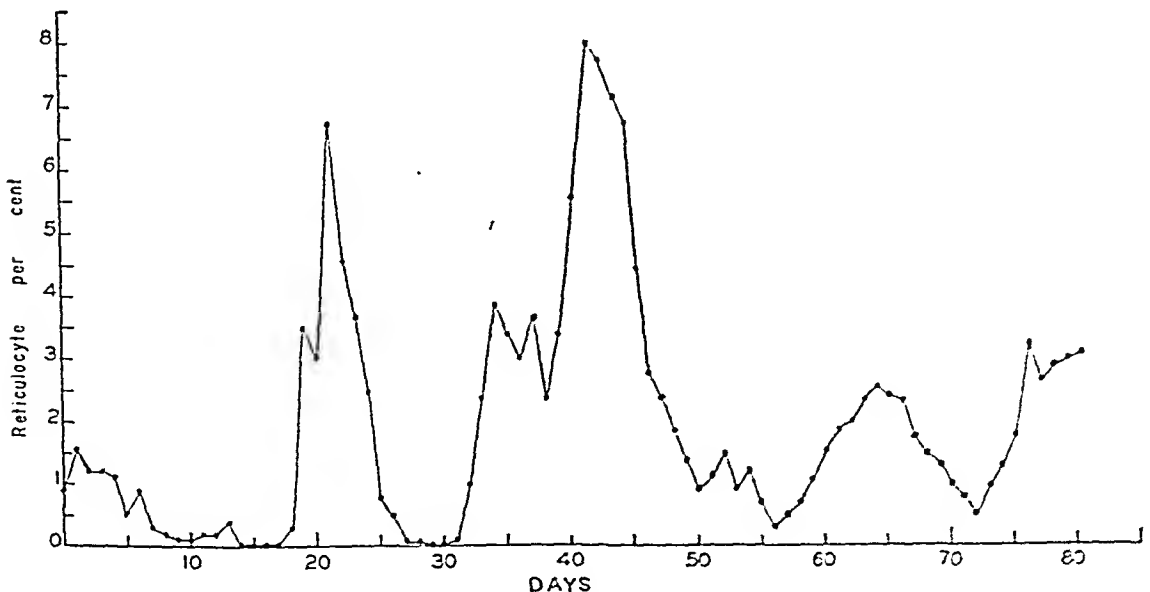


FIG. 14. GUINEA PIG NUMBER 114, ♂, 535 GRAMS. GOLDBERGER DIET. MARKED SPONTANEOUS SWINGS IN RETICULOCYTE LEVELS.

curing normally in a non-anemic guinea pig is obviously entirely different in significance from the abnormal megaloblastic hyperplasia seen in pernicious anemia patients in relapse. It may be recalled here that once previously the impression that a megaloblastic marrow in a normal animal rendered that species suitable for liver assay purposes resulted in much futile work on pigeons.<sup>2</sup>

It would appear at present that there is no physiological basis for the development of a specific reticulocytosis to liver in animals with normal blood, and in our experience the guinea pig is no different in this respect from the other species of normal laboratory animals which have failed to yield a bioassay method for anti-pernicious anemia substances.

A search of the literature revealed only two reports apparently confirming the usefulness of the normal guinea pig as a test animal. The first study, by Landsberg and Thompson (12), was based on only 6 animals, and no controls were mentioned. In 4 of their 6 animals the pre-injection reticulocyte levels would render them unsuitable for the method here under consideration. The report of Miller and Rhoads (16) deserves special mention because these workers implied confirmation of Jacobson's results and stated that "increases in the number of circulating reticulocytes which are similar in degree, duration, and time of occurrence to those obtained by Jacobson may be induced by potent, anti-anemic substances which are orally as well as parenterally administered." A careful analysis of their data, however, reveals that their results differ from Jacobson's in important respects. In the first place, the height of reticulocytosis obtained by Miller and Rhoads was definitely greater than Jacobson's. The latter's positive responses were under 3.4 per cent in 90 per cent of 193 experiments, whereas, in Tables II and III presented by Miller and Rhoads, at least 5 responses out of the 11 recorded were over 3.4 per cent, and in one

experiment all 3 animals reached 5 per cent or over. Since the method deals essentially with low reticulocyte values, such a difference in one of its fundamental phenomena appears significant. More important, however, is the fact that Miller and Rhoads' reticulocyte responses did not occur until the *seventh to thirteenth day* after the initiation of treatment, while Jacobson's criterion of a positive response demands that it occur *within six days*. The importance of this difference is well illustrated in Jacobson's Table VII (9) which shows that animal 256 did not respond within six days to a presumably impotent material given orally. This animal was then immediately given a second mixture, and this time a reticulocyte response did occur within the required period of six days. On the basis of these time relationships, this so-called positive response, coming as it did on the twelfth day after the *original* impotent material, was regarded by Jacobson as evidence of potency of the second and impotency of the first preparation. Miller and Rhoads, on the other hand, might readily have regarded the first preparation as potent, because they accepted such late reticulocyte responses as positive. Indeed, inasmuch as all the positive responses obtained by Miller and Rhoads occurred *after* the first week, these same positive responses would, if judged by Jacobson's criteria, be called negative responses.

These discrepancies in the degree and time of appearance of the reticulocytosis cannot be ascribed to differences in dosage. Jacobson (9, Table V) noted no influence on the magnitude of the reticulocyte rise after the injection of the minimal effective dose as compared with 6000 times this quantity. Furthermore, he noted a tendency for the responses to occur earlier, if anything, within the six-day observation period when larger amounts of liver were given. Our own interpretation of these data is that here again one is encountering nothing more than the usual non-specific and spontaneous reticulocyte fluctuations of normal guinea pigs.<sup>3</sup>

<sup>2</sup> It is of interest to note that very recently Jones (11) investigated the bone marrow of the guinea pig, pigeon, and rat. He found that the red cells of these marrows "belong to the definitive or normoblastic series, and they are identical with those of the marrow of the normal human adult, which does not possess megaloblasts." There was "—no morphologic evidence for a megaloblastic bone marrow in the guinea pig," and no similarity to the megaloblastic marrow of pernicious anemia.

<sup>3</sup> In a recent personal communication C. P. Rhoads has written, "Further experimentation has advanced incontrovertible evidence that guinea pig reticulocytes may increase to a level of over 2 per cent from a variety of causes unassociated with the administration of substances effective in the treatment of pernicious anemia. Experiments are under way in an attempt to elucidate

We have carefully observed the four precautions listed by Jacobson in his discussion (9) in regard to the diet and environment of the pigs, the high degree of accuracy in reticulocyte counting, the selection only of animals stable during a preliminary control period for liver injection, and the use of liver extract of a high degree of therapeutic potency. Despite meeting these and the other criteria of the method, on the basis of our experience we are forced to conclude that the normal guinea pig exhibits, in respect to his reticulocytes, such a capricious behavior that the use of this species for bioassay of liver extract is at present unsuitable and hazardous.

The prediction is ventured that a satisfactory laboratory assay animal will not be obtained until it is possible to produce experimentally a clinical picture closely simulating the pernicious anemia of man. Whether the anemia of swine produced by Miller and Rhoads (15), or the anemia associated with liver cirrhosis obtained in rats by Higgins and Stasney (6), will provide the long-sought laboratory test animal remains a question for further experimentation.

#### CONCLUSION

The normal adult guinea pig shows considerable and unpredictable spontaneous fluctuations in reticulocyte levels. These variations are of such a nature as to render this normal animal unsuitable for assaying the potency of materials effective in pernicious anemia.

The authors are grateful to Mrs. Helen Goodman for her invaluable technical assistance.

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the cause of spontaneous variations in numbers of reticulocytes; such spontaneous variations demand that the greatest care be used in employing the guinea pig as a test for anti-anemic substances."





PROCEEDINGS OF THE TWENTY-EIGHTH ANNUAL MEETING OF THE  
AMERICAN SOCIETY FOR CLINICAL INVESTIGATION  
HELD IN ATLANTIC CITY, N. J., MAY 4, 1936

READ BEFORE JOINT SESSION

*Streptococcus Immune Bodies in Rheumatic Fever and in Comparable Control Groups.* By JOHN R. MOTE (by invitation) and T. DUCKETT JONES, Boston, Mass.

The results of the antibody response to streptolysin, fibrinolysin, and precipitins to the C, carbohydrate fraction, and the D, E, K, and P fractions of the hemolytic streptococci were studied in 90 cases of scarlet fever, in 56 cases of pharyngitis in non-rheumatic subjects, in 262 rheumatic recurrences, and in 218 upper respiratory infections in rheumatic subjects without recurrences.

It was found that over 90 per cent of the cases with scarlet fever and pharyngitis had an immune response to one or more of the antigens used.

Of the patients with rheumatic recurrences 40 per cent had no preceding respiratory infection, and 33 per cent had no antibody response to any antigen studied.

Of the 218 respiratory infections which are commonly followed by rheumatic fever in rheumatic subjects, 36.4 per cent had no antibody response to any of the antigens used. The great similarity of the percentage of cases having an antibody response whether a recurrence developed or not, is striking. However, the antibody response in the group without recurrences was in general not so great as in the group with recurrences.

In view of these findings it is concluded that although respiratory infections, especially those associated with the hemolytic streptococcus, are common precursors to rheumatic fever recurrences there must be some other factor than the streptococcus to account for the facts as found.

*Bacterial Endocarditis Following the Ingestion of Bacteria.* By R. O. MUETHER (by invitation) and R. A. KINSELLA, St. Louis, Mo.

In a previous communication a method for the production of experimental bacterial endocarditis was discussed. Since that time we have had the opportunity of seeing endocarditis with septicemia develop in a number of animals which were fed a mixture of dog "chow" and living bacteria, following the mechanical damage of a valve of the heart.

*Methods.* Twelve healthy dogs, varying in size from six to twelve kilograms were operated under intravenous barbitol anesthesia in the manner previously described. Upon regaining consciousness, a mixture of "chow" and *Streptococcus viridans* (10 cc. 24 hour broth culture), was offered and this food was taken daily until a positive blood culture was obtained, or until seven to twenty-one days had elapsed. Following this procedure five animals developed septicemia and died, showing at autopsy the pathological picture of septicemia and bacterial endocarditis. The gastro-intestinal tract showed no macroscopic lesions.

An attempt to determine whether or not the organisms

recovered from the infected dogs were identical with those which had been fed was made. Sugar reactions and agglutination tests were done for this purpose.

The bacteria which we used were obtained from a case of subacute bacterial endocarditis. These bacteria, as well as those recovered, were planted in mannite, lactase, sucrose, raffinose, inulin, and salicin.

The agglutination tests were done with rabbit's serum obtained from rabbits injected subcutaneously, and intravenously with living bacteria obtained from the original human source. Injections were begun with one-half cc. given subcutaneously, and were increased a half cc., every other day until 3 cc. had been given. One-half cc. was then injected intravenously and increased by one-half cc. every other day until five cc. were given. Ten days were allowed to elapse after the last injection, and preliminary titration showed that the serum agglutinated the original organisms in a dilution of 1/1280. The bacteria recovered from the animals were then tested.

*Results.* The five dogs which did not yield a positive blood culture after feeding were finally injected intravenously with *Streptococcus viridans* as indicated in the table. All then gave positive blood cultures. One died three days after the positive blood culture was obtained, and the others are under treatment.

Three dogs which made up a total of twelve died within twenty-four hours of their first feeding and were discarded.

The sugar reactions were remarkably constant. Only one discrepancy was found. These bacteria failed to ferment mannite at first, but subsequent tests gave uniform results. Two cultures of bacteria obtained from the patient also yielded consistent results.

The agglutination tests were also consistent. Strong agglutination was obtained in all dilutions up to and including 1:1280. The bacteria used in this experiment grew smooth, and thus facilitated the use of agglutination tests.

*Conclusions.* On the basis of information available, it would seem that a septicemia may develop following feeding of a mixture of dog "chow" and living bacteria to dogs whose heart valves have been recently damaged. When these bacteria lodge upon the damaged heart valve they produce vegetations which resemble bacterial endocarditis produced by intravenous injection. Endocarditis and septicemia so produced is rapidly fatal.

*Experimentally Induced Nephritis in Rats. Functional and Clinical Studies.* By LEE E. FAER and JOSEPH E. SMADEL (introduced by Homer F. Swift), New York, N. Y.

Sera obtained by immunizing rabbits with emulsion of perised rat kidney induced in rats a nephritis charac-

terized histologically by progressive glomerular and tubular destruction. Twenty-four rats were observed for preliminary periods of three weeks to one month while blood and urine chemical studies, and urea clearances, were done. These rats were then injected with varying amounts of anti-kidney serum, and followed for periods up to 313 days. The animals uniformly developed nephritis. Some died in the acute stage; others were killed at intervals to supplement histological data obtained from those dying spontaneously. Of those surviving the acute stage, the majority developed a progressive chronic nephritis; a few recovered, some completely. Albuminuria, casts, plasma protein deficit, lipemia and slight to marked depression of urea clearance, without hypertension or retinal changes, characterized the acute nephritis; and during this phase, animals became markedly edematous, with ascites, hydrothorax and generalized subcutaneous edema. Animals with chronic lesions and in terminal phases of the disease showed marked reduction in urea clearance with blood urea nitrogen up to 350 mgm. per cent, anemia, hypertension, moderate plasma protein deficit, malnutrition, transient edema, cessation of growth and slight retinopathy. In many respects this induced disease simulated Bright's disease in man.

*The Effect on the Kidneys of Bilateral Splanchnicectomy in Patients with Hypertensive Vascular Disease.* By R. H. FREYBERG and M. M. PEET (introduced by F. N. Wilson), Ann Arbor, Mich.

The concentrating ability of the kidneys, urea clearance, proteinuria, and formed elements of the urine sediment were measured in patients with hypertensive vascular disease ("essential" or "malignant" hypertension) before and at varying lengths of time after resection of both greater and lesser splanchnic nerves. In thirty-two patients with adequate postoperative study the results were in general as follows. In those patients (53 per cent) who had a significant and maintained decrease in blood pressure following splanchnicectomy, the urinary abnormalities decreased or disappeared and the renal function, if it had been impaired, improved—in several cases it became entirely normal. When hypertension was lowered in patients having normal renal function prior to operation the kidney efficiency remained normal. When hypertension was not favorably influenced by this operation, renal function remained unchanged or became gradually worse, although proteinuria was frequently less.

The results of these studies show that the hypertension is not compensatory to measurable renal damage; that marked impairment of renal function may accompany hypertensive vascular disease; and that striking improvement of function follows relief of the hypertension.

*The Action of Protamine Insulin in Normal and Depancreatized Dogs.* By ROBERT B. KERR (introduced by C. H. Best), Toronto, Canada.

The action of protamine insulin, which was first described by Hagedorn, Jensen, Krarup and Wodstrup, has

been studied in normal and depancreatized dogs. The protamine was prepared from the sperm of the "Spring Salmon" (*Onchorhynchus tshawytscha*) by Scott and Fisher of the Connaught Laboratories.

The prolongation of the action of insulin by the addition of protamine has been demonstrated in normal dogs. The blood sugar has been lowered for 12 to 30 hours following large doses of protamine insulin without hyperglycemic convulsions.

It is possible to keep depancreatized dogs in good condition and "sugar free" on a liberal diet of two feedings per day with one dose of protamine insulin daily. Glycosuria is much increased when one dose of regular insulin, of the same number of units, is substituted for protamine insulin. The blood sugar of depancreatized dogs remains at a much more uniform level throughout the 24 hours when the animal is receiving protamine insulin than when regular insulin is being given.

The addition of zinc salts to insulin has been found to prolong its action in dogs in confirmation of the work of Scott and Fisher in rabbits. In further confirmation of the work of these authors, ash free preparations of insulin and protamine show considerably shorter duration of action than do the same preparations to which a small amount of a zinc salt has been added.

The possibility of toxic effects resulting from the administration of protamine has been studied.

*The Effect on the Human Electroencephalogram of Various Drugs Which Influence Nervous Activity.* By F. A. GIBBS and E. L. GIBBS (by invitation) and W. G. LENNOX, Boston, Mass.

The fluctuations in electrical potential which can be lead off the head are modified by certain drugs in therapeutic doses. The most definite changes attend the use of convulsants, sedatives and anesthetics. Drugs primarily affecting the autonomic nervous system produce little change.

Sedatives cause changes similar to those observed in normal sleep. In place of the fast rather steady activity characteristic of the waking state, there are slow large voltage fluctuations with occasional bursts of fast activity and also short periods in which there are almost no fluctuations. If sedation is so heavy that the patient cannot be aroused, the bursts of fast activity disappear, the slow components become slower and of larger voltage, and almost continuous. Ether produces first a decreased voltage and increased frequency and later large voltage waves without fast components. Electroencephalography may, therefore, be of value in controlling depth of anesthesia and sedation.

Convulsants produce large voltage disturbances such as are seen in epilepsy; the frequency may be fast or slow. In patients having frequent petit mal seizures, bromide or phenobarbital in doses insufficient to change normal electrical activity prevents or disorganizes the pattern of the discharge which characterizes the seizure.

*Familial Lipemia of Undetermined Origin.* By L. EMMETT HOLT, JR., and (by invitation) FRANCIS X. AYLWARD and HARRY G. TIMBUS, Baltimore, Md.

An eleven year old girl was observed who from the age of four years had suffered from periodic acute attacks with abdominal pain and rigidity, vomiting, fever and collapse—the attacks lasting one to four days and subsiding spontaneously. On one of these occasions the abdomen had been explored without finding an explanation for the symptoms. There was also a history of psoriasis, worse in the summer time, and of chronic ulcers on the legs appearing in the summer following minor trauma, resisting all treatment but healing spontaneously in the winter. On examination the patient was somewhat undernourished; in addition to some characteristic lesions of psoriasis she showed two chronic granulating ulcers on the leg; the liver and spleen were enlarged, the former being four fingerbreadths and the latter two fingerbreadths below the costal margin. The eye grounds showed typical lipemia retinalis; the blood serum was milky and contained more than seven per cent fat, the excess consisting almost entirely of neutral fat.

A careful study failed to reveal any evidence for any of the familiar causes of lipemia: the carbohydrate metabolism was normal; renal function and liver function—the latter studied by carbohydrate and dye tests—were normal; there was no evidence of poisoning, of anemia or of endocrinopathy. Studies of the respiratory metabolism revealed no difficulty in burning fat. It was not possible to determine whether the lipemia resulted from difficulty in removing fat from the blood or from excessive mobilization of depot fat.

While the patient was under observation a number of typical abdominal attacks occurred; at the termination of each one it was found that the lipemia had disappeared, the blood fat being only slightly above the normal value. It would then gradually increase toward a critical level of about eight per cent when another attack would occur and the process would be repeated. Attempts were made to reduce the blood fat by means of choline, insulin, liver extract, thyroxin, anterior pituitary extract and by transfusions with little success. Despite these measures the blood fat would rise; the rise was, however, somewhat less rapid on the choline period. When the fat intake was reduced to fifteen grams a day and five grams a day of choline given by mouth in addition, the blood fat gradually fell and the lipemia disappeared. The liver and spleen decreased markedly in size, the patient's general appearance was improved, and she began to gain weight. It is questionable whether this improvement is being maintained on the low fat diet without choline.

An investigation of the patient's family revealed that a younger brother of two and a half years had an enlarged liver and spleen, lipemia of the retina and a blood fat of three per cent. He had not had attacks similar to those in the patient.

It is suggested that we are dealing here with a familial anomaly of lipid metabolism, analogous to Gaucher's disease, Niemann-Pick disease and xanthomatosis, with the

exception that in this condition it is neutral fat rather than other lipids which are not normally disposed of and which therefore tend to accumulate in the phagocytic cells of the reticulo-endothelial system, causing hypertrophy of the liver and spleen.

*Observations on the Gastric Secretion of Man.* By LEON SCHIFF, Cincinnati, Ohio.

During the past four and one half years over six hundred fractional gastric analyses have been made on one individual under similar conditions, both with and without histamine. On two occasions, each lasting many weeks, the patient developed post-histamine achlorhydria. Analyses of the gastric juice made during the phases of achlorhydria are compared with those made during phases of normal secretion. These include determinations of volume, acidity, chloride, base, pepsin and the "intrinsic" anti-anemic factor.

It is hoped that these observations may throw some light on the mechanism of the secretion of gastric juice.

*The Manner of Removal of Aqueous Solutions from Joints.* By GRANVILLE A. BENNETT, FREDERIC W. RHINELANDER, 2ND. (by invitation), and WALTER BAUER, Boston, Mass.

Previous studies have shown that proteins are removed from normal joints only by way of the lymphatics. Similar information concerning the removal of simple aqueous solutions is necessary in order better to interpret the manner of fluid exchange in normal and pathological joints.

The manner of absorption of aqueous solutions from cat knee joints was determined by recording the blood pressure changes following the intra-articular injection of vasoconstrictor and vasodilator solutions. The total amount absorbed was calculated by assaying the joint washings at the end of each experiment.

Adrenalin apparently produced sufficient local vasoconstriction to prevent its absorption in detectable amounts. The prompt systemic effects following injection of pituitrin and pilocarpine indicated rapid absorption via the capillaries.

Acetyl choline ("mecholyl" acetyl-beta-methylocholine chloride, Merck) was employed in most experiments. Its absorption from normal joints was demonstrable within 30 seconds. This early effect and its inhibition by intra-articular injection of adrenalin likewise indicated absorption via the capillaries.

Exercise increased absorption under all conditions. Previous removal of synovial fluid or slight inflammation did not significantly affect absorption. Absorption was strikingly increased from acutely inflamed joints.

In two of nine experiments done on eserized cats, acetyl choline was demonstrable in thoracic duct lymph samples at the end of 45 and 60 minutes.

Thus it would appear that the chief route of absorption from a joint of an aqueous solution such as acetyl choline is by way of the subsynovial capillaries.

*Studies on the Relief of Pain by Counterirritation.* By GEORGE D. GAMMON (by invitation) and ISAAC STARR, JR., Philadelphia, Pa.

The authors induced pain in themselves by skin irritants and by the subcutaneous injection of hypertonic NaCl, applied heat, cold, electrical and mechanical counterirritation, and studied the relief afforded. The effectiveness of certain agents varied greatly in the two types of pain, e.g. heat relieved pain after NaCl injection but increased that from irritants.

The application of any counterirritant was usually followed by relief of short duration; when pain returned the removal of the counterirritant was also followed by relief. In certain instances rhythmic application and removal of the counterirritation relieved severe pain which constant application could not control.

Experimentally induced pain and its relief have been further analyzed in man by observing the changes caused by experimental alteration of the circulation and, with the aid of Dr. D. W. Bronk, in anesthetized cats by records of action potentials in cutaneous nerves. Skin injury evokes a discharge of rapid impulses which is greatly increased by heat and which shows little decline due to adaptation.

The studies made on induced pain have been repeated on 40 patients with pain of various origin. Visceral, cutaneous, and neuritic pain respond differently to different counterirritants, a fact which suggests a means of differential diagnosis.

#### READ BEFORE SECTION A

*The Circulation in Lobar Pneumonia with Special Reference to Pulmonary Edema.* By W. M. HITZIG, F. H. KING, J. G. M. BULLOWA (by invitation), and ARTHUR M. FISHBERG, New York, N. Y.

Seventy-five patients were studied at the height of lobar pneumonia. Arm-to-tongue circulation time (saccharin) was normal in 67, prolonged in 8. Of these 8, 4 had preëxistent heart disease. Arm-to-lung time (ether) paralleled arm-to-tongue time. Normal circulation time was observed within 30 minutes of death. Venous pressure was normal in 66, elevated in 9. Of those with elevated venous pressure, 3 had preëxistent heart disease, in 2 it was secondary to alterations in respiratory mechanics due to pulmonary edema, and 1 had singultous. Circulating blood volume (congo red, 65 cases) was normal. Eight patients were studied at the height of pulmonary edema (7 fatalities). All had normal circulation time. Six had normal venous pressure, 2 slight elevation.

*Conclusions.* Heart failure sufficient to retard blood flow is very rare in pneumonia without preëxistent cardiac disease. This supports the view that routine digitalization is inadvisable.

The normal circulation time indicates that pulmonary edema in pneumonia is not due to heart failure. Previous investigators have found evidence of pulmonary hypertension and generalized increase in capillary per-

meability in pneumonia. It is suggested that these factors participate in the pathogenesis of pulmonary edema in pneumonia.

*Vasomotor Collapse.* By SOMA WEISS and (by invitation) ROBERT W. WILKINS, Boston, Mass.

Sodium nitrite in small amounts which induce no appreciable changes in the hemodynamics in the horizontal position of the body disturbs the cardiovascular adjustment essential for the upright position. As a result, progressive manifestations of circulatory collapse develop within 20 to 60 minutes, accompanied by symptoms and signs of collapse identical with those observed in disease. During the first and symptom-free stage the small pulse pressure, rapid heart rate and thready pulse of 140 to 150 per minute are associated with increased arterial and venous tone, but with unchanged height of the effective venous column of the blood and with unaltered arteriovenous oxygen difference and blood flow. Later, when pallor, ashy-gray color of the skin, "cold-beaded" perspiration, lassitude and drowsiness appear, the pulse pressure is but 6 to 10 mm. Hg, and as a result of the failing adjustments, the effective venous column and blood flow fall, and the oxygen difference increases. During this stage yawning, sighing and deep respiration represent adjusting functions, which may temporarily bring the blood flow back to normal. Finally a stage is reached when the markedly decreased blood flow, which may be 25 per cent of the normal level in the hand and presumably in the brain, accentuates the parasympathetic manifestations, and complete circulatory collapse and unconsciousness develop. Following the return of the body to horizontal position the symptoms promptly disappear, and the circulation becomes normal within 1 or 2 minutes. Comparative observations indicate that the circulatory changes in syncope, collapse and shock are similar, the most obvious difference being the time element.

*Evaluation of Medicinal Treatment in Angina Pectoris.* By JOSEPH E. F. RISEMAN and MORTON G. BROWN (introduced by Herrman L. Blumgart), Boston, Mass.

The efficacy of medicinal therapy in angina pectoris was evaluated in thirty patients by observing changes in the clinical frequency of attacks and by quantitating the amount of effort possible under standardized conditions before inducing an attack.

Evaluation by clinical means indicated that improvement followed placebo pills as frequently as any other form of medication. The standardized exercise tolerance test revealed no significant improvement following placebo pills, sodium bicarbonate, potassium iodide, or oral tissue extract. About one-third of the patients failed to benefit by any of the fifteen different drugs employed.

Nitroglycerine given prophylactically enabled about two-thirds of the patients to undertake approximately 100 per cent more exercise before inducing anginal pain. In many patients this prophylactic effect was evident for as long as one hour. About one-half of the patients were

improved by aminophyllin or quinidine sulphate; one-third by erythrol tetranitrate, codeine sulphate or atropine sulphate; and one-fifth by sodium nitrite, theophyllin calcium salicylate or dinitrophenol. Phenobarbital was rarely of benefit. Digitalis frequently caused a marked increase in pain and reduction in the amount of exercise possible.

The duration of action and the optimal dosage were also determined. In several individuals nitroglycerine, grains 1/500, taken every hour rendered the patients free of attacks in daily life. Complete disappearance of cardiac pain following medication was rare. Aminophyllin and nitroglycerine caused an increase in exercise tolerance of about 20 per cent to 100 per cent.

*The Capillary Supply of the Rabbit Heart in Experimental Hypertrophy.* By R. A. SHIPLEY and LOUISE J. ZSCHIESCHE (by invitation), and J. T. WEARN, Cleveland, Ohio.

If, in cardiac hypertrophy, there should be an increase in the mass of muscle tissue without a corresponding increase in the number of accompanying capillaries, a point might be reached when the tissue would suffer from a deficiency in the exchange of metabolic substances.

A quantitation has been made of the number of capillaries per unit of tissue in hearts with hypertrophy produced experimentally in rabbits. Hypertrophy resulted in an increase in the diameter of the muscle fibers with a consequent decrease in the concentration of capillaries in the cross sectional plane. During normal growth it was found that enlargement of fibers was accompanied by a birth of capillaries so that the concentration of capillaries per unit of tissue remained constant.

*The Coronary Flow in Dilated Human Hearts.* By WILLIAM B. KOUNTZ, St. Louis, Mo.

The work of Anrep has established that in a state of contraction the coronary flow of a dog's heart is considerably reduced. Because of the thinness of the muscle walls and the irritability of the dog's heart it is impossible to study the effect of dilatation on the coronary flow. The heart of man, however, has a relatively large muscle mass which enables it to maintain an adequate blood pressure even though the diastolic volume is greatly increased. For this reason we have attempted to establish the effect of dilatation of the human heart on the coronary flow.

Hearts were revived and made into a heart-lung preparation. The coronary flow was measured by placing cannulae in the coronary arteries and perfusing from a reservoir, in the top of which was inserted an instrument for measuring the amount of air displaced by blood flowing into the arteries. The volume of the heart was measured by an oncometer placed over the heart after the heart-lung preparation had been completed.

After a record of the coronary flow and the heart volume had been taken the organ was caused to dilate by stopping the coronary flow and producing asphyxia. The heart could be seen to dilate, and when its volume increased to the point where it could just sustain a systolic

blood pressure of 120 mm. of mercury, the coronary system was opened and the flow and heart volume were measured.

The effect of drugs upon the coronary flow and cardiac volume was then determined by injection into the perfusing system. Three groups of drugs were used in this study. The first consisted of three different digitalis preparations, which were chosen because it has been shown that they decrease the diastolic volume of the normal heart and also decrease the coronary artery flow. The second group of drugs were known coronary dilators of which histamine and sodium nitrite were the chief ones used. These drugs are also known to increase the diastolic volume of the normal heart. The third group consisted of the xanthine compounds, of which theobromine was chiefly used. This drug dilates the coronary arteries but does not change the cardiac volume.

When the compounds of digitalis were injected into the coronary arteries of the dilated heart there was a rather marked decrease of the diastolic volume of the heart and an increase in the flow into the coronary arteries. Theobromine had very little effect on the coronary flow. On the other hand, histamine, which increased coronary flow in the normal heart, definitely decreased it in dilated hearts.

These experiments, we believe, tend to emphasize two points, one physiological and the other clinical. From a physiological standpoint they serve to emphasize that changes in the heart from its normal diastolic state, whether by dilatation or contraction, produce a decrease in the coronary flow. Anrep has explained the decreased flow in the contracted heart by assuming that there is a squeezing and narrowing of the capillary bed. We suggest that the decreased flow with dilatation is due to a linear traction on the blood vessels of the heart which produces a narrowing of the capillaries and consequent decrease in the vascular bed.

From a clinical standpoint, not only the state of the coronary vessels but also the degree of contraction or dilatation of the cardiac chambers must be considered. No single coronary dilator can be expected to exert a favorable action in all cases.

*Concerning a New Concept of the Genesis of the Electrocardiogram.* By L. N. KATZ and (by invitation) A. BOHNING, M. ROBINOW, I. GUTTMAN, H. KOREY and F. OCKO, Chicago, Ill.

Studies carried out in this laboratory during the past few years have lead us to a new concept of the genesis of the electrocardiogram. The problem concerns the electrical field created by the heart. This can be divided into three phases: (1) the sequence and orientation within the heart of the electrical stresses set up, (2) the spread of the electrical currents generated by the heart in the body as a whole, and (3) the influence on the electrical field which results from the variable electrical conductivity of the tissues immediately adjacent to the heart.

While considerable attention has been paid in the past to the first two aspects of the problem, little thought has

been given to the last phase. Not all regions of the heart are in contact with good conductors. It is obvious that those that are have a decided advantage over other regions of the heart in their influence on the electrical field. The various regions have been defined by us. Our work indicates that the variable electrical conductivity of the tissues in contact with the heart exerts a much greater influence on the electrocardiogram than the electrical conductivity elsewhere in the body.

In view of the above, it is apparent that changes in the electrocardiogram can result from variations in the relative position of the electrical conductors to the heart. This may be caused either by alterations in position or shape of the conductors adjacent to the heart or by changes in the position or shape of the heart itself. As a consequence, regions of the heart which normally exert a great influence on the electrocardiogram may become "silent," while other regions previously "silent" may now exert a prominent effect. We are presenting the experimental evidence which supports this concept.

Our results indicate that the electrocardiogram is not a summation of events occurring in all parts of the heart but is primarily a summation of events in those regions which are in contact with good electrical conductors. Other regions play a relatively minor rôle. This concept readily explains all the abnormalities of the electrocardiogram including some hitherto obscure ones. It also offers a rational basis for the use of precordial leads.

*Changes in the Vaginal Smears of the Menopause during Periods of Spontaneous Symptomatic Relief.* By EPHRAIM SHORR and (by invitation) GEORGE N. PAPANICOLAOU, New York, N. Y.

We have previously reported<sup>1</sup> that adequate amounts of estrin administered to women with the menopausal syndrome changed the vaginal smear from that of the menopause to the leukopenic follicular type characteristic of the normally menstruating woman just prior to ovulation. This change was accompanied by symptomatic relief.

We have since observed four cases in which temporary spontaneous relief of their menopausal symptoms occurred. Studies of the vaginal smears revealed the fact that a change to the follicular type had spontaneously taken place at these times. There then followed a gradual regression of the smear to the menopausal type, with the reappearance of symptoms.

There was a complete similarity between the changes in smears and symptomatology during the periods of spontaneous relief, and those observed during the administration of ovarian follicular hormone. These observations suggest that the induction of the follicular smear type is a rational objective in the replacement therapy of the menopausal syndrome.

<sup>1</sup> Papanicolaou, G. N., and Shorr, E., *Proc. Soc. Exper. Biol. and Med.*, 1935, 32, 585.

*The Relation of the Anterior Pituitary to Liver Glycogen Production and Utilization.* By EDWARD D. CHURCHILL, Boston, Mass.

Rabbits six to eight weeks old were totally hypophysectomized. Baby rabbits were used because of their established reactions. Operative approach was through the nasopharynx, avoiding brain damage. The liver glycogen, blood sugar and lactate levels were studied under various conditions. Ten to twelve hours following glucose feeding a subnormal blood sugar plateau of 75 to 60 mgm. is reached. This plateau lasts four to six hours during which time the liver glycogen store gradually disappears. When the liver glycogen reaches a trace, 0.03 to 0.05 per cent, an abrupt fall in blood sugar starts. This fall is remarkably constant, occurring at the rate of about 10 mgm. per hour until convulsive levels are reached. It closely resembles the fall in blood sugar following hepatectomy. It is suggested that in the absence of the anterior pituitary, endogenous production of carbohydrate is so impaired that when the liver glycogen store is depleted the hypophysectomized animal is virtually hepatectomized. Intravenous glucose not only restores the blood sugar level but is readily deposited as liver glycogen. Intravenous sodium lactate in contrast to glucose is utilized but slowly. Adrenalin releases lactate but its failure to raise blood sugar is apparently in the lactate utilization.

*Hypoglycemia. A Problem in Carbohydrate Metabolism.* By STANLEY E. DORST, Cincinnati, Ohio.

An investigation of the varying response of adult malnutrition to insulin therapy reveals a group presenting an unusual problem in carbohydrate metabolism. These patients are undernourished and fail to show any permanent improvement under adequate dietary treatment. The glucose tolerance test shows a curious curve. Beginning at a subnormal fasting level the introduction of glucose either orally or parenterally is not followed by the expected increase in blood sugar, instead the values seldom rise above 100 mgm. per cent, nor do they drop below 60 even after 5 hours. No glucose is lost in the urine. This is definitely not the curve of hyperinsulism but resembles somewhat the configuration associated with hypothyroidism. These patients, however, have not had low metabolic rates.

With insulin therapy, paradoxically, the curve rapidly returns to the expected figures. There is rapid gain in weight, nervousness, asthenia and general malaise disappear. When insulin is withdrawn both the chemical and clinical picture reappears to respond again when insulin is resumed. Some of these patients have now been followed over 3 years.

All of our undernourished patients who have shown an adequate response to insulin therapy have fallen in the hypoglycemic group. Those who have a normal response to the ingestion of glucose have not shown clinical improvement on insulin therapy. These facts invite speculation and permit the introduction of certain theoretical concepts.



*Potential Variations of the Precordium and of the Extremities in Myocardial Infarction.* By CHARLES E. KOSSMAN and CLARENCE E. DE LA CHAPPELLE (introduced by Dr. Arthur C. DeGraff), New York, N. Y.

The potential variations of six precordial points and of the right arm, the left arm, and the left leg were recorded by the method of Wilson, Johnston, Macleod, and Barker in a series of 69 patients with myocardial infarction. In ten individuals who came to necropsy the gross and microscopic extent of the lesion was determined. The precordial electrocardiograms were similar to those obtained by Wilson and his associates in a study of experimental infarction with direct leads by the same method. The form of the initial and final ventricular deflections of the precordial curves depended upon the age and extent of the infarct, and the proximity of the exploring electrode to dead muscle, normal muscle, or combinations of the two. A true intrinsic deflection (chief upstroke) was absent when the exploring electrode was placed, (1) at a point on the precordium close to heart musculature which microscopically showed acute necrosis or replacement fibrosis of all or most of the muscle cells; (2) at the right sternal edge, the left sternal edge, and the tip of the ensiform in cases with extensive infarction of the left ventricular apex, the apical two-thirds of the anterior wall of the left ventricle, and the apical two-thirds of the anterior half of the interventricular septum, but with normal right ventricle; and (3) at the same points as in (2) in a case with extensive perivascular and interfascicular fibrosis of the entire left ventricle and septum due to coronary atherosclerosis and previous rheumatic myocarditis.

#### READ BEFORE SECTION B

*Morphologic Changes in the Blood Associated with Experimentally Produced Hepatic Damage.* By M. M. WINTROBE and (by invitation) H. B. SHUMACKER, JR., Baltimore, Md.

Acute and chronic hepatic damage have been produced in dogs and in rabbits by the administration of carbon tetrachloride. Following the initial doses of this drug, or after the dosage was abruptly increased, polycythemia developed. After injury to the liver was caused repeatedly and a chronic condition resembling cirrhosis was produced, anemia developed. This was often normocytic in type but in a number of instances in which the hepatic damage was particularly severe and of long duration, macrocytic anemia occurred. In the animals in which the latter type of anemia developed, the bone marrow was found to be hyperplastic.

The morphologic changes which occurred in the blood of the animals in these experiments resemble those which are found clinically in association with hepatic disease. The possibility that the macrocytic anemia may be the result of faulty storage of antianemic principle is considered.

*The Coagulation of Blood by Proteolytic Enzymes.* By H. EAGLE and (by invitation) T. HARRIS, Philadelphia, Pa.

Trypsin and papain both coagulate whole blood and plasma. The latter acts directly on the fibrinogen, converting it to an insoluble modification resembling fibrin. Trypsin, however, has no direct effect on the fibrinogen, but converts prothrombin to thrombin.

The coagulation of blood by trypsin is therefore analogous to physiological coagulation, since the enzyme acts like the physiologic system *calcium + platelets*, in initiating the production of thrombin. There is reason to believe that calcium and platelets (or calcium and tissue extracts) together may constitute a proteolytic enzyme which either combines with or hydrolyzes prothrombin to form the physiologic coagulant, thrombin.

*The Mechanism of Iron Transportation: Its Significance in Iron Utilization in Anemic States of Varied Etiology.* By CARL V. MOORE (by invitation) and C. A. DOAN, Columbus, Ohio.

The mechanism by which iron is transported in the blood stream has consistently defied experimental definition. Three forms of blood iron have so far been recognized: (1) hemoglobin iron, (2) plasma iron, and (3) "easily split-off" iron, so termed because it may be "split-off" from hemoglobin by weak acids (Barkan). In association with, or as one of, the latter two forms, iron transportation is probably effected.

The present study emphasizes the extreme lability of plasma iron; the literature indicates the relative constancy of "easily split-off" iron; and both these forms are apparently independent of fluctuations in the hemoglobin content. In clinical states characterized by rapid destruction (*e.g.*, congenital hemolytic icterus), and under conditions of diminished iron utilization (*e.g.*, aplastic anemia and untreated pernicious anemia), the plasma iron has been found to be distinctly higher than its normal value of 75 to 150 micrograms per cent. Whenever the bone marrow was stimulated to unusually active erythropoiesis as in liver induced remissions in pernicious anemia, there was a precipitous fall in the plasma iron level coincident with the reticulocytosis—iron presumably being withdrawn from the plasma more rapidly than it could be mobilized from the various storage depots. Iron deficiency states, whether of dietary, absorptive, or hemorrhagic etiology, have been attended by lower than normal plasma iron values. Oral administration of large single doses of iron has been followed by a temporary rise of the plasma iron to values as high as 500 micrograms per cent.

"Easily split-off" iron has been identified with the function of iron transport (Barkan), plasma iron being considered the medium of direct exchange with the tissues. However, in view of the relative constancy of its value, the known fluctuation in the iron content of the various storage depots, and the above described lability



of plasma iron in terms of iron intake and utilization, it is logical to conclude that iron is transported primarily as plasma iron. The physiological significance of "easily split-off" iron remains yet to be defined.

*Treatment of Pernicious Anemia with Congo Red.* By HALSEY BARKER (introduced by C. P. Rhoads), New York, N. Y.

Mazza and Zolezzi, as well as Mermod and Dock, have reported that Congo red solution parenterally administered is effective in the treatment of pernicious anemia. Experiments designed to test the efficacy of Congo red in maintaining cases of pernicious anemia in remission have been made. A group of patients was kept symptom-free and at high blood levels by liver extract administered intramuscularly at 2-week intervals. Liver extract was then discontinued and 0.5 per cent Congo red solution was administered weekly intravenously in 20 cc. amounts. In every instance a progressive increase in the mean corpuscular volume and the color index resulted, as well as a decrease in the number of erythrocytes and a recurrence of symptoms. Two cases of sprue recurred similarly. One case of pernicious anemia at a low blood level failed to improve on the daily administration of a small amount of liver extract. The evidence indicates that Congo red is not an effective substitute for liver extract in the treatment of pernicious anemia.

*The Coproporphyrin of the Urine and Feces under Normal and Pathological Conditions.* By CECIL JAMES WATSON (introduced by H. A. Reimann), Minneapolis, Minn.

1. Coproporphyrin I (configuration not corresponding to hemoglobin) was isolated from normal urine, and in increased amounts from the urines of the following instances: (a) Hemolytic jaundice of acquired type. (b) Fever due to lung abscess and empyema. (c) Cincophen cirrhosis of the liver.

2. Coproporphyrin III (configuration corresponding to hemoglobin) was isolated from the urines of three cases of lead poisoning.

3. Coproporphyrin I was isolated from normal feces and in increased amount from the feces of patients with hemolytic jaundice. Coproporphyrin I was also obtained from the feces of a case of lead poisoning during the same period in which the urine yielded only the isomeric coproporphyrin III.

Coproporphyrin III is regularly excreted in the urine in lead poisoning, and probably owes its formation to a peculiar disturbance in hemoglobin metabolism. Coproporphyrin I, whose formation is by independent synthesis, occurs in the normal urine and feces in small amount. It is increased in the feces in hemolytic jaundice and pernicious anemia (previous report) and in the urine in conditions exhibiting evidence of liver dysfunction, notably urobilinuria.

*Deficiency of Plasma Magnesium and Excess of Plasma Potassium in Essential Epilepsy.* By ARTHUR D. HIRSCHFELDER and (by invitation) VICTOR G. HAURY, Minneapolis, Minn.

Having reported previously a group of clinical cases with low plasma magnesium in all of whom neuromuscular twitchings or convulsions were present, we have determined plasma magnesium in cases of essential epilepsy at the Minnesota Colony for Epileptics (Cambridge, Minn.), using a slight modification of our Titan Yellow colorimetric method. In normal individuals we found plasma magnesium 1.8 to 2.4 mgm. (average  $2.11 \pm 0.11$  mgm.) per 100 cc. plasma, ultrafiltrable magnesium (through number 300 sheet cellophane) 0.9 to 1.3 mgm. (average  $1.08 \pm 0.12$  mgm.). We found low plasma magnesium during convulsions in 65.6 per cent of 32 severe epileptics and in 40 per cent of 35 less severe epileptics. The lowest magnesium (1.2 to 1.5 mgm.) occurred only in the most severe cases. In 78 out of 79 cases the magnesium was normal in the interim between periods of convulsions when the patients had been free from convulsions for at least several days, but magnesium was sometimes low just before or just after convulsions.

Plasma potassium (average normal 20.85 mgm. K per 100 cc. plasma) was more than double the normal during convulsions in 71.9 per cent of the severest cases and in 56 per cent of the moderately severe cases, and was but slightly above normal in the milder cases.

The potassium/magnesium ratio was above normal in all 67 cases during the convulsions, more than double the normal in 71.9 per cent of the severest cases and in 20 per cent of the moderately severe, but only slightly above normal in the mildest cases. The Ultrafiltrable K/Ultrafiltrable Mg ratio was increased more intensely than the Total K/Total Mg ratio.

There were no critical levels of either magnesium, potassium or K/Mg ratio at which convulsions were certain to take place or to be absent.

Since Hirschfelder has shown that potassium salts antagonize the narcotic effects of magnesium salts it is probable that low plasma magnesium, high potassium, and especially high potassium magnesium ratios are important contributing factors in bringing on the convulsions of essential epilepsy.

Plasma calcium was always normal, phosphate was sometimes moderately increased (to 6 to 8 mgm.) during the convulsions but much less frequently and less intensely than the potassium; hypoglycemia was never present before, during or after the convulsions, but blood glucose increased moderately during convulsions.

Oral administration of 2 grams  $MgCl_2$  four times daily for three months did not diminish the frequency or intensity of the convulsions nor did oral administration of 2 grams KCl four times daily increase them. Rectal enemas of 30 grams epsom salt usually and intramuscular injection of 1 gram epsom salt almost always inhibited convulsive seizures.

*The Effect of High Protein Diet and Intraperitoneal Injection of Ringer's Solution on Renal Hypertrophy after Unilateral Nephrectomy in Rats.* By PAUL L. STIER (by invitation) and J. M. HAYMAN, JR., Cleveland, Ohio.

The observation that rats ingesting a diet containing large amounts of protein show an increase in kidney weight has been made repeatedly. These animals drink much water and excrete a large volume of urine. The question, therefore, arises whether the increase in kidney weight is related to the excretion of the larger amounts of fluid. After unilateral nephrectomy, white rats of approximately the same age were divided into three groups. One group was fed a low (18 per cent) protein diet, the second a high (67 per cent) protein diet, and the third the low protein diet plus daily intraperitoneal injections of Ringer's solution in amounts comparable to the urine volume of animals on the high protein diet. After 40 days, the animals were killed, and the increase in kidney weights compared. The rats on the low protein diet showed a mean increase of 52 per cent in kidney weight, those on the high protein diet a mean increase of 116 per cent, and those receiving Ringer's solution 66 per cent. The excretion of increased volumes of urine is apparently not an important factor in the renal hypertrophy following high protein diets.

*The Concentration of Urate in Cells and Plasma as a Function of pH.* By JOHN H. TALBOTT and (by invitation) JANE M. SHERMAN, Boston, Mass.

The distribution of urate between cells and plasma as a function of the hydrogen ion concentration has been studied in the blood obtained from 3 patients with gout. In all instances venous blood was used, samples of which were equilibrated at 37.5° C. in a water bath at varying tensions of CO<sub>2</sub>. The hydrogen ion concentration was calculated according to the Henderson-Hasselbach equation. In all of the experiments reported at this time the concentration of urate in plasma, cells and whole blood was determined colorimetrically according to the method of Benedict.<sup>1</sup> Chloride and water concentrations were determined simultaneously with urate in plasma and cells. The distribution of urate and chloride between plasma and cells was calculated according to the equation,

$$\frac{\frac{(H_2O)_c}{(H_2O)_s} \cdot \frac{(H_2O)_c}{(H_2O)_s}}{\frac{(H_2O)_c}{(H_2O)_s}}$$

In 3 experiments the urate concentrations were determined at varying hydrogen ion concentrations in blood to which no uric acid was added. With increasing acidity there was a diminution in concentration of urate in the plasma and an increasing concentration of urate in the cells. The average diminution in plasma urate concentration over a pH change of 1.0 was 2.3 mgm.

<sup>1</sup> Benedict, S. R., and Behre, J. A. J. Biol. Chem., 1931, 92, 161.

per 100 cc. This percentile change is large and approximately ten-fold greater than the change in water concentration.

In 3 other experiments 8 to 10 mgm. of uric acid per 100 cc. were added to whole blood. In these, the average urate ratio between cells and plasma at pH = 7.40 was 0.58. At the same pH the chloride ratio was 0.64. The change in the urate ratio with pH followed closely the change in the chloride ratio.

In one experiment the urate and chloride ratios were determined for oxygenated and reduced whole blood. At pH = 7.80 the spread between oxygenated and reduced whole blood for the urate ratio was 1.0 and for the chloride ratio was 0.8. At pH = 6.90 the spread for the urate ratio was 0.2 and for the chloride ratio was 0.2. These data are interpreted to mean that the distribution of urate between cells and plasma is affected by the Gibbs-Donnan law of equilibrium.

*Comparative Biological Effects of Neutron Rays and X-Rays.* By JOHN H. LAWRENCE and ERNEST O. LAWRENCE (introduced by Francis G. Blake), New Haven, Conn.

In a previous paper it was demonstrated that neutron rays are biologically active, as determined by studying the fall in the number of white blood cells in rats after irradiation. Other animals were irradiated with high voltage x-rays. Using the roentgen unit, as a measure of dosage for both neutrons and x-rays, the neutron seemed to be more effective than x-rays. This was not surprising, since the ionization in tissues by neutrons is probably produced by recoil protons, which are heavy charged particles, when compared with electrons.

In the present work we have studied further the comparative effects of neutrons and x-rays on tissues, as measured by the lethal dose for mice and rats, and the lymphopenia produced in the rat. The results indicate that neutrons are much more biologically active than x-rays.

*Studies on the Relationship of the Hypophysis to Hematopoiesis.* By OVID O. MEYER and (by invitation) GERTRUDE E. STEWART, and HAROLD P. RUSCH, Madison, Wis.

It has been found that hypophysectomy in rats is followed by a persistent subnormal reticulocyte count in the peripheral blood. When these hypophysectomized animals are exposed to a reduced atmospheric pressure, no reticulocytosis occurs though sharp increase in the controls is regularly observed. Exposure to reduced pressures soon after hypophysectomy is followed by polycythemia comparable to that of the controls but none occurs after a 25-day postoperative period. The bone marrow does not become hyperplastic as in the controls. The results in animals that have been splenectomized before hypophysectomy are analogous to those with spleen intact.

Though the stimulus of oxygen want and the administration of liver extract do not produce an increase in

reticulocytes, the administration of large doses of antuitrin (growth hormone) is followed by marked reticulocytosis in every animal, reaching a maximum of 15 per cent in one instance. There is, however, persistent anemia unless thyroxin is administered with the growth hormone.

Thyroidectomized and orchidectomized rats respond to oxygen want in a manner analogous to that of normal animals.

*Quiet Breathing in a Closed Circuit, with Progressively Decreasing Oxygen Concentrations: Effect upon Composition of Expired Air.* By D. W. RICHARDS, JR., and (by invitation) H. C. A. LASSEN and A. COURNAND, New York, N. Y.

When a normal resting subject breathes through a small closed circuit with progressively decreasing oxygen concentration, it is found after several minutes that the nitrogen concentration of an expired air sample, taken at the end of an expiration, is about 1 per cent less than the nitrogen concentration of the immediately preceding inspired air. The calculated oxygen utilization is also small, with R.Q. values well over 1.0.

When the same system is arranged so that the inspired air either maintains a constant composition, or has a steadily increasing oxygen concentration, the nitrogen concentration of expired air is then about 1 per cent greater than that of inspired air, as one would expect.

The explanation is probably this. Each inspired breath is added to a relatively large volume of (functional) residual air. Each expired breath is a mixed sample of air from this residual lung volume, and contains air from a number of earlier inspired breaths. With progressively decreasing inspired oxygen, the expired samples thus tend to have relatively higher oxygen concentrations, and lower nitrogen concentrations, than those of the immediately preceding inspired air.

In the breathing system described, therefore, the complete and equal mixing of an inert gas, which has often been assumed, does not actually exist.

#### READ BY TITLE

*Anticomplementary Wassermann Reactions Associated with Hyperproteinemia, particularly in Lymphogranuloma Inguinale and Multiple Myeloma.* By ALEXANDER B. GUTMAN and (by invitation) RUSSELL D. WILLIAMS, New York, N. Y.

Williams and Gutman called attention recently to the occurrence of hyperproteinemia in lymphogranuloma inguinale. Values of 8.0 to 11.2 per cent, due chiefly to hypereuglobulinemia, were found in 16 of 20 cases examined. It was noted, further, that the Wassermann reaction was reported as anticomplementary in 22 per cent of 74 Frei-positive patients, as compared with approximately 1 per cent in the general hospital population. This association suggested the possibility of a similar relationship in other diseases in which hyperproteinemia occurs.

It was found that in 2 of 3 cases of multiple myeloma

with hyperproteinemia, but in none of 4 without hyperproteinemia, anticomplementary Wassermann reactions were obtained (cf. Magnus-Levy). We have observed, also, anticomplementary Wassermann reactions associated with hyperproteinemia in occasional cases of tuberculous lymphadenitis, non-specific infections, lymphosarcoma, pregnancy. In kala-azar, moreover, in which hyperproteinemia is the rule, Mu and Huie report anticomplementary Wassermann reactions in 16 per cent of their cases.

There is evidence, presumptive and experimental, to indicate that increased serum globulin, particularly in the euglobulin fraction, is one of the important factors associated with non-specific fixation of complement in man.

Persistent non-specific fixation of complement, whether observed in the course of the Wassermann reaction or in the gonococcal-complement fixation test, may, in our experience, occasionally prove of diagnostic value.

*Influence of Nutrition and of Pancreatectomy on Resistance to Experimental Septicemia.* By RUSSELL RICHARDSON (introduced by C. N. H. Long), Philadelphia, Pa.

This study was designed to correlate the recognizable changes in blood and tissue chemistry occurring in experimental diabetes under varying conditions of nutrition with the duration of life and the degree of bacteremia following a standard infection with staphylococcus aureus.

The experiments have been conducted on 45 cats: 25 depancreatized, 11 on liberal diet and insulin, 14 on restricted diet with minimal dosage of insulin; 20 control cats, 13 on unlimited diet, 7 on restricted diet.

The procedure has been: (1) preliminary period on the selected diet in the control and depancreatized animals. (2) Examination of blood for concentration of sugar and serum for concentration of CO<sub>2</sub> and refractive index, and of muscle and liver for glycogen content; in some experiments measurements were made of cholesterol, albumin, total protein and water content of serum and of glycogen content of skin and spleen. (3) Inoculation of controls and depancreatized cats with standard dose of staphylococcus aureus. (4) At daily intervals until death, blood culture with bacterial count and repetition of the examinations of blood and tissue chemistry. (5) Record of the duration of life after inoculation.

The correlations observed and inferences that can be drawn will be presented.

*Renal Physiology in Lobar Pneumonia.* By LEE E. FARR and THEODORE J. ABERNETHY (introduced by Rufus Cole), New York, N. Y.

The immediate and delayed effects of pneumococcus pneumonia upon renal function were studied in twenty-five unselected patients. Urea clearances were done on admission, at crisis, five days postcrisis and at intervals following discharge for six months. Addis sediment counts were done on admission, at crisis and six days postcrisis. Frequent retinal examinations, daily blood

blood pressure may be of palliative value in moderate or severe cases when organic change precludes the hope of markedly lowering the blood pressure.

Our limited experience with laminectomy and anterior root section leads us to believe that the operative mortality and the chance of postoperative complications is too great for any but the most hopeless cases.

*The Behavior of Human, Bovine and Avian Tubercle Bacilli in Media Containing Sulphur.* By BURGESS L. GORDON and (by invitation) R. C. ROSENBERGER and A. PROSKOURIAKOFF, Philadelphia, Pa.

Preliminary studies suggest that the addition of 4 mgm. of elementary sulphur to 100 cc. of Corper's medium prevents the growth of tubercle bacilli. It appears also that sulphur exerts a bacteriostatic influence.

*Valometric Studies of the Circulation in a Sharply Defined Portion of the Human Finger Tip.*<sup>1</sup> By W. A. SODEMAN and G. E. BURCH (by invitation) and R. H. TURNER, New Orleans, La.

Using a sphygmoplethysmograph sensitive to 0.1 cubic millimeters, we have measured changes in volume of a sharply defined portion of the human finger tip in response to the pulse and to changes of position of the finger from heart level to a position 45 cm. above and 45 cm. below heart level and changes due to sudden application of pressure within a sphygmographic cuff about the upper arm while the finger was in an elevated position. The part studied reached its minimum volume while in the elevated position following pressure occlusion of the arm vessels. For the purpose of our calculations, the finger tip was assumed to be bloodless when it reached its minimum volume. All volumes above this base line were accepted as representing the total blood volume of the part at that stage of the observation. The volume of soft tissue was also determined, thus enabling the expression of blood volume in terms of per cent of soft tissue.

The subjects studied have all been adult males, ranging from 20 to 70 years of age, including the following: 17 normal individuals, 6 suffering from diastolic hypertension, 9 showing arteriosclerosis alone, 9 with arteriosclerosis and diastolic hypertension, and 2 with congenital clubbing of the fingers.

Our data indicate: A. In patients with arteriosclerosis without diastolic hypertension, the mean volume of pulsations was approximately that of the normal; the mean total blood volume was reduced; and the volume of pulsations, expressed as per cent of total blood volume, was increased. The extremes of individual variations of the total blood volume in patients with arteriosclerosis without diastolic hypertension were greater than in the normal group.

B. In patients with diastolic hypertension, the volume

of pulsations and total blood volume at heart level were reduced. In the elevated position the total blood volume was approximately that of the normal group but the volume of pulsations was again reduced. In the lowered position the total blood volume was reduced while the volume of pulsations approximated that of the normal group. In all positions the volume of pulsations, expressed as per cent of the total blood volume, was about the same as normal. The range of individual determinations of the volume of pulsations, total blood volume, and volume of pulsations expressed as per cent of total blood volume at heart level, was greatly limited as compared to the normal and arteriosclerotic groups.

C. In patients with arteriosclerosis and diastolic hypertension, the mean volume of pulsations, total blood volume and volume of pulsations, expressed as per cent of total blood volume, in general fell between the values obtained in patients with these diseases separately.

D. In a patient with localized Raynaud's phenomenon (dead finger) with marked atrophic changes in the left index (affected) finger, distinct changes were noted at room temperature as compared to the corresponding normal (right index) finger. The involved finger showed a reduced total blood volume in all positions, out of proportion to the reduction in the total soft tissue volume. The volume of pulsations was slightly reduced at heart level and definitely so at the elevated and lowered positions. The volume of pulsations expressed as per cent of total blood volume was greater in the involved finger at heart level and the lowered position, but was less at the elevated position.

E. In two cases of congenital clubbing of the fingers, the volume of pulsations was markedly reduced in all positions and was affected negligibly by position. The total blood volume and the volume of pulsations expressed as per cent of total blood volume were relatively and absolutely reduced in all positions as compared to all other groups.

*The Effect of Intramuscular Adrenalin upon the Heart-rate in Psychoneurotic Patients.* By STANLEY COBB and (by invitation) JACOB E. FINESINGER, Boston, Mass.

Continuous records of heart rate were made by means of a cardiograph<sup>1</sup> on thirteen patients with psychoneuroses of various types. The patients were made to stand up and lie down at regular intervals before and after the intramuscular injection of adrenalin (Parke-Davis) in doses varying from  $\frac{1}{2}$  to 1 cc., 1 to 1,000. The mean heart rate in beats per minute was read from the records. Before adrenalin the standing rate always exceeded the lying rate, the difference between the two varying from 15 per cent to 120 per cent. In addition to the accelerating effect upon both standing and lying rates, intramuscular adrenalin caused the lying rates to approach the standing rates and tended to obliterate at

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stroke volume multiplied by the systolic arterial pressure in proper units is a close approximation of the work per beat of the left ventricle in this valvular lesion.

Calculations based on estimations of the amount regurgitated with various hypothetical stroke volumes and arterial pressures in human aortic insufficiency give a conception of the relative work of the left ventricle in that disorder.

*Undernutrition in the Treatment of Coronary Artery Disease (Particularly Thrombosis). Effect on the Basal Metabolism and Circulation.* By A. M. MASTER, H. L. JAFFE and S. DACK (introduced by George Baehr and B. S. Oppenheimer), New York, N. Y.

The effect of low caloric intake on basal metabolism and circulatory dynamics was investigated in 29 cases of coronary thrombosis and 13 of angina pectoris. An 800 calorie diet, 80 grams carbohydrates, 50 proteins and 30 fat, was maintained for 3 to 12 months. The control basal metabolic readings lay within  $+10$  and  $-10$ . In 74 per cent, a significant fall in basal metabolism of 15 to 35 per cent occurred in two to four weeks. The causes for failure of the metabolism to fall significantly were repeated angina pectoris, fever, pulmonary disease and cardiac insufficiency. The factors influencing metabolism in heart disease were studied. An increase in caloric intake effected a rise in metabolic rate. The loss of weight associated with the drop in metabolism averaged 6 per cent of initial body weight. The blood chemistry remained unchanged; no myxedema appeared.

The effect on circulation of the low caloric intake with its resultant drop in metabolism consisted of slowing of pulse rate and fall in blood and pulse pressures. The cardiac output (determined in one case) was reduced 33 per cent. All these factors resulted in reduction of the work of the heart. The blood velocity and vital capacity remained unchanged.

*Clinical Observations Bearing on the Selection of Cases for Surgical Treatment of Essential Hypertension with Results in Twenty-Four Cases.* By ROBERT STERLING PALMER and (by invitation) REGINALD H. SMITHWICK, Boston, Mass.

Clinical observations of 224 cases of abnormally elevated blood pressure followed for varying periods during the past five years are reported. A special attempt has been made to find cases among young adults and cases in which an elevated blood pressure was an incidental finding, in order to study the development of essential hypertension of varying degrees of severity at different ages.

Considering the group as a whole, cases fall into mild, moderate, and severe. The mild cases have lower pressures which are variable, often returning to normal, are free from organic change, tend to occur in younger patients, possibly more often in males. Moderate cases have higher pressures, variable but less apt to return to normal, often have asymptomatic organic changes, and occur at somewhat older ages. Severe cases have the high pressures which, though just as variable as the mild

or moderate, never return to normal, have marked organic changes with symptoms of circulatory insufficiency in brain, eyes, heart, or kidneys, and tend to occur at older ages with one notable exception: *the most severe cases, in the accelerated malignant phase occur under 50 years of age and in our experience most of them in females before the menopause.* We have observed occasional cases pass from one stage to another, the first being marked by functional nervous symptoms sometimes related to difficulties of emotional nature and accompanied by a variable hypertension, which later though still variable, attained a higher level. In one patient the course from a mild variable hypertension to intractable malignant hypertension and death occupied three years.

As a working hypothesis we suggest that essential hypertension may be related to a constitutional susceptibility or irritability of the sympathetic adrenal system. Precipitating factors in the latter state may be nervous tension and endocrine abnormalities in females. Clinical observation indicates that many cases pass through a mild predominately vasomotor, stage offering an opportunity for cure or alleviation by surgery of the sympathetic nervous system when medical management fails to halt the progress.

In view of our clinical observations and with this working hypothesis we have employed various surgical procedures in 24 cases; in severe cases because of distressing symptoms and the hopeless prognosis, in moderate cases because observation indicated progress, in mild cases because the pressure was not controlled by medical measures and because the patients fell into a prognostically bad group (females of child-bearing age).

Our results are as follows: Six patients (5 severe, 1 mild) were subjected to alcohol injection of the splanchnic nerves. A temporary (days to weeks) fall in blood pressure was obtained. A disproportionate symptomatic improvement was noted. Adrenal denervation or adrenalectomy was done in 4 severe cases and 1 mild case of essential hypertension. A temporary fall in blood pressure was the result. Symptomatically improvement was noted and in one case marked improvement in the eye grounds was observed. Three severe cases were subjected to splanchnic resection without any permanent benefit. Finally 9 cases (6 severe, and 3 mild) have had supradiaphragmatic splanchnic resection. Of the six late cases a material reduction in blood pressure has been observed in 3 with decreased fluctuation in 4, but no return to normal. All 3 of the mild cases have shown material lowering of the blood pressure, 2 to normal levels, stabilization of the blood pressure in 2, decreased fluctuation in the third. One case of malignant hypertension has had laminectomy and anterior root section recently, and it is too soon to report results.

We have not taken symptomatic improvement as a criterion of success because we seek to prevent the more or less remote results of continued arterial hypertension. Because incidental operations such as cholecystectomy cause a temporary fall in blood pressure and symptomatic improvement. However, diminution in fluctuation of the

blood pressure may be of palliative value in moderate or severe cases when organic change precludes the hope of markedly lowering the blood pressure.

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least for a short time the percentage difference between the lying and standing rates observed before adrenalin. In six cases the percentage difference between lying and standing rates was reduced to zero or to a minus value (that is, the standing rate was equal to or lower than the lying rate). This reduction occurred between six and thirteen minutes after adrenalin. In four cases the lying and standing rates returned to their original values at the end of the experiment (twenty-three to ninety-two minutes after adrenalin). In three additional cases the standing rates alone had practically returned to their preliminary values. In the remaining experiments which lasted from forty to one hundred minutes after adrenalin the standing and lying rates were greater at the end of the experiments than before the administration of adrenalin.

*Ascorbic Acid in the Treatment of Thrombocytopenic Purpura.* By DAVID K. MILLER (by invitation) and C. P. RHOADS, New York, N. Y.

The effect of the administration of ascorbic acid has been studied in a group of patients with thrombocytopenia associated with bleeding into the skin and from the mucous membranes. No effect has been seen in cases which were diagnosed leukemia, aleukemic leukemia, or aplastic anemia. In four cases of apparently idiopathic thrombocytopenic purpura a persistent rise in the number of thrombocytes and complete relief of symptoms followed the administration of ascorbic acid. In two such cases the excretion of ascorbic acid was measured quantitatively in order to test the relative degree of saturation with vitamin C. In both instances clinical improvement and an increase in the number of thrombocytes were associated with an increased urinary output of ascorbic acid. The results suggest that ascorbic acid may be therapeutically effective in certain cases of thrombocytopenic purpura.

*Direct Measurement of Blood Flow through the Kidneys of Unanesthetized Dogs.* By A. BLALOCK, T. R. HARRISON and (by invitation) M. F. MASON, Nashville, Tenn.

For the purposes of studying renal blood flow and oxygen consumption, the following technique has been used. At a preliminary operation, the two spermatic veins, the left adrenal vein and any other branches (except the right adrenal vein) draining into the renal veins or into the vena cava near the entrance of the renal veins have been tied. A metal marker is tied to the inferior vena cava about three centimeters below the renal veins. Several days after the animals recovered entirely a large thin-walled metal cannula is passed under novocaine anesthesia from the right external jugular vein down through the superior cava and into the inferior cava as far as the ligature. Several different methods were employed to prevent leakage and back flow around the cannula. The most satisfactory device has been a modified Morowitz cannula carrying two balloons, one of which lies below and the other above

the renal veins. The position of the cannula has been checked by fluoroscopic examination. By inflation of these balloons it is possible to obtain, through the cannula, all the blood flow through the kidneys during a measured period of time. Analyses of the oxygen contents and capacities of this renal venous blood and of arterial blood allow one to calculate the renal oxygen consumption. Data for these functions in normal animals, in animals after unilateral nephrectomy, and following the administration of various drugs, are presented.

*Arteriovenous Fistula: Diagnosis from the Oxygen Content of the Blood of the Regional and Proximal Deep Veins.* By BAYARD T. HORTON and (by invitation) GRACE M. ROTH and ELIZABETH McCLAY, Rochester, Minn.

The term "arteriovenous fistula" is used to designate any abnormal communication between an artery and a vein by means of which arterial blood enters the vein without passing through a capillary bed. The immediate receiving group of veins show an increase in oxygen content, which depends upon the amount of arterial blood shunted into them by way of the fistula.

Determinations of the degree of admixture of arterial and venous blood in the regional and proximal deep veins was carried out in sixty cases of arteriovenous fistula. In fifty of these cases the condition was congenital, and in six of these the fistula was intracranial. In the remaining ten cases the fistula was acquired.

Blood was collected under oil, and the oxygen capacity and content were determined by the Van Slyke gasometric method. In the intracranial group, blood was obtained from the internal jugular veins, and the mean oxygen saturation was greater than 90 per cent. In cases in which the fistula was in the upper extremities the average saturation was 89 per cent, in the lower extremities, 82 per cent.

When surgical exploration was carried out, similar postoperative determinations were made to evaluate the results of surgery.

*The Influence of Laparotomy on the Gastric Motor Mechanism.* By GEORGE M. CURTIS and (by invitation) LOUIS E. BARRON, Columbus, Ohio.

Using the balloon and kymograph method we have investigated the motor response of the stomach to various abdominal operations. Extended serial observations are reported on one patient with cholelithiasis for which cholecystectomy was done, and on three patients with indirect inguinal hernia. Studies were made in the morning about fourteen hours after the preceding evening meal. The usual duration of each experimental observation was approximately five hours. Adequate control studies were made prior to and again subsequent to each surgical procedure. The results were uniform.

In the patient with cholelithiasis, normal gastric motility was observed during the control period. It was possible to investigate the gastric motor response during biliary colic. This revealed continuous gastric motility

throughout the observation period. Severe contractions were at times observed. Studies were resumed subsequent to cholecystectomy which was accomplished under gas-ether anesthesia. Daily observations revealed that there ensued intense gastric motility persisting for about ten days. This hypermotility was associated with epigastric distress, interpreted by the patient as "gas pains." The motility subsequently returned to normal.

Observations were likewise made on three patients prior to, during, and subsequent to herniorrhaphy. The motility during the preoperative control period was essentially normal. Studies were made on the morning of operation to note the effect of stress on the gastric motor mechanism. In certain instances these revealed hypermotility associated with mild epigastric distress. Kymographic records of gastric motility during herniorrhaphy conducted under spinal anesthesia demonstrated complete gastric inhibition. This persisted for about twenty-four hours, at which time the stomach regained its tonus. Forty-eight hours subsequent to herniorrhaphy there was gas-ether anesthesia. Daily observations revealed that this persisted for about ten to twelve days. Associated with the gastric hypermotility the patients complained of epigastric distress which they designated as "gas pains." The gastric motility of all patients eventually returned to normal.

*Neurological Findings in Patients with Rheumatoid Arthritis.* By CHARLES L. SHORT and (by invitation) ALFRED O. LUDWIG, Boston, Mass.

Towards the end of the nineteenth century, the neural origin of rheumatoid arthritis was upheld by many observers. The symmetry of the joint lesions, the atrophy of muscles and skin, and other sensory and motor phenomena including bulbar involvement were cited in evidence. Inconstant pathological changes in the cord and peripheral nerves were found. With the advent of bacteriology, this aspect of the disease has been largely overlooked.

We have attempted to reopen the subject in the present study. Symptoms and signs attributable to the nervous system have been recorded in a large percentage of 250 carefully studied patients. Examination of the spinal fluid in 50 patients in all stages has revealed no constant deviation from the normal. Such fluid has been injected intracisternally in cats, without subsequent evidence of disease of the nervous system or joints. Anterior horn cell degeneration has been found at autopsy in one patient who showed bulbar and spinal neuronitis before death and changes in the peripheral nerves in four.

It is hoped that further studies will enable us to interpret the significance of these findings. Greater interest is desirable in this neglected but important phase of rheumatoid arthritis.

*The Etiology of the Anemia of Iron Deficiency.* By CLARK W. HEATH and (by invitation) ARTHUR J. PATEK, JR., Boston, Mass.

The iron requirement, or the demand for iron by the organism, should logically be considered first in the eti-

ology of iron deficiency. A reduction of the supply of iron, in the form of reduced dietary intake, malabsorption, or both, is an important and often necessary factor, but theoretically cannot alone produce iron deficiency.

Study of the iron requirements for normal growth (in particular increasing blood volume) and menstruation discloses important facts which explain the appearance of iron deficiency anemia at different periods of life. In infancy, and after puberty total annual iron requirements are greatest and at these times iron deficiency frequently appears. The data explain the appearance of iron deficiency (1) equally in the two sexes during infancy; (2) in girls at puberty, when the annual requirement for iron is proportional to that for a normal pregnancy; (3) in women more commonly than in men, since in women the total requirement until the time of the menopause is about four times that of adult men. In adult men iron deficiency anemia has been observed by the authors only following blood loss. Pathological bleeding and pregnancy have been observed to be important additional factors in the etiology of iron deficiency anemia in women. Chronic, often obscure, pathological blood loss is practically always present in "idiopathic" hypochromic anemia.

*Observations on the Mechanism of Recovery in Gonococcal Arthritis.* By WESLEY W. SPINK (by invitation) and CHESTER S. KEEFER, Boston, Mass.

A study of the mechanism of recovery in patients with gonococcal arthritis has included an analysis of the bactericidal action of whole blood on the gonococcus. Although the whole blood is capable of killing gonococci in large numbers, this bactericidal power resides in the serum. Washed leukocytes or heated serum possess little or no killing power.

The blood of patients with gonococcal metastatic lesions, as arthritis, tends to have a higher bactericidal power for the gonococcus than normal controls. Similar studies are being carried out with patients having only localized lesions such as urethritis. An attempt is being made to study the virulence of individual strains by this method.

The bactericidal power of a normal individual was not increased following repeated injections of a gonococcal filtrate. Intradermal and subcutaneous injections of a gonococcal vaccine in another normal control was followed by no increase in bactericidal action of the whole blood. Intravenous gonococcal vaccine resulted in a slight but temporary increase in the bactericidal power of a patient with gonococcal arthritis. The intravenous injection of a stock anti-gonococcal horse serum caused an immediate and marked increase in the killing power of the blood of another patient, but one of short duration.

*Observations on the Mechanism of Muscular Twitchings in Uremia.* By T. R. HARRISON and (by invitation) M. F. MASON and H. RESNIN, Nashville, Tenn.

Muscular twitchings in uremic subjects have been ascribed in the past to phosphate retention, resulting in a



deficit of calcium ions in the blood. The following evidence indicates that the twitchings are of central rather than peripheral origin:

1. Intracisternal administration of inorganic phosphorus to dogs causes marked twitchings, similar doses being entirely ineffective intravenously.

2. When large doses of inorganic phosphorus are given intravenously, the onset of twitchings is usually delayed until the content of phosphorus in the cerebrospinal fluid increases.

3. In dogs with experimental anuria following double nephrectomy or bilateral ureteral ligation, the development of muscular twitchings is related to increase in the phosphorus content of the cerebrospinal fluid rather than that of the blood.

4. Both in experimental and in clinical uremia muscular twitchings may be alleviated by the intracisternal administration of calcium salts in doses which are ineffective when administered intravenously.

Accumulation of inorganic phosphorus in the cerebrospinal fluid is not the only cause of muscular twitchings, which may occur when the phosphorus content of the blood and cerebrospinal fluid is at a normal level. In such instances, increase in the amount of guanidine-like substances in the blood has been demonstrated. Since these compounds produce marked twitchings when administered to normal animals it is believed that their presence may account for the neuromuscular irritability which occurs occasionally without phosphorus accumulation.

In the terminal stages of uremia, twitchings may be absent in spite of marked increase in the phosphorus content of the body fluids. Under these conditions high values for the free phenols in the blood have been found. We have shown that the previous administration of free phenols will prevent the muscular twitchings which are ordinarily produced by the intracisternal injection of phosphorus.

In conclusion it is believed that accumulation of phosphorus and of guanidine in uremic subjects tend to produce twitchings, and that retention of phenol derivatives tends to prevent them. Their presence or absence depends on the balance of these opposing factors.

*The Lipiduria of Bright's Disease: Observations on the Urinary Excretion of Cholesterol, Lipid Phosphorus and Fatty Acids.* By MAURICE BRUGER with the technical assistance of WILLIAM PEARLMAN (introduced by H. O. Mosenthal), New York, N. Y.

In previous reports from this laboratory, we showed that in the urine of patients with Bright's disease, the cholesterol excretion paralleled the protein excretion.<sup>1</sup> This problem has been pursued further and at this time, studies on the cholesterol, lipid P and fatty acid content of 27 urines obtained from nine patients with Bright's disease are reported. In two patients, serial determina-

tions of 13 specimens of blood and urine, collected simultaneously, were carried out over a period of more than two months.

The concentration of urinary cholesterol varied from a trace to 1.97 mgm. per cent, lipid P from 0.007 to 0.134 mgm. per cent, and fatty acids from 0.07 to 4.78 mgm. per cent. In general, the concentration of the urinary lipids paralleled one another closely, the cholesterol/lipid P and the fatty acid/cholesterol ratios remaining fairly constant. Apparently, the degree of proteinuria is not only an indication of the amount of cholesterol excreted in the urine, but of lipid P and fatty acids as well. Serial blood and urine studies in two cases indicated, however, that alterations in the lipid P and fatty acid content of the blood were accompanied, with few exceptions, by similar changes in the concentration of these lipids in the urine, whereas the association between blood and urine cholesterol was not so evident.

*Enterogenous Cyanosis with Met- and Sulphhemoglobinemia; Clinical and Spectrophotometric Studies.*<sup>1</sup> By HENRY H. HENSTELL (by invitation) and WILLIAM DAMESHEK, Boston, Mass.

Spectrophotometric observations with the Hardy recording spectrophotometer in a woman with bouts of cyanosis and diarrhea revealed the presence of both met- and sulphhemoglobin. Since no etiological agent was discernible and alternating diarrhea and constipation were present, the diagnosis of enterogenous cyanosis was made. Of 36 cases which have been reported since 1902, 23 showed sulphhemoglobin, 12 methemoglobin, and one both pigments.

An intimate relationship was evident between the appearance of diarrhea and subsequent appearance of abnormal pigment in the red cells. Disappearance of pigment always resulted in anemia which in turn was followed by reticulocytosis. The diarrhea was dependent upon a proctitis characterized by inflammation and pinpoint ulcerations of the rectal mucosa. A bland diet with milk seemed curative, but attempts at induction of the original disorder with high protein and high sulphur diets were unsuccessful at a time when the rectal mucosa was relatively normal in appearance.

This is the second recorded case in which both met- and sulphhemoglobin were demonstrated and the first in which spectrophotometric studies were secured. Our studies further demonstrated the relationships of met- and sulphhemoglobin to each other and to the intestinal pathology. The blood studies revealed another cause for reticulocytosis.

*Combined Cord Degeneration without Anemia: A Case Report with Studies Bearing on the "Intrinsic Factor" of Castle.* By WALTER LINCOLN PALMER and (by invitation) ROBERT T. PORTER, Chicago, Ill.

In this paper an untreated case of subacute combined cord degeneration of three years' duration, without

<sup>1</sup> J. Clin. Invest., 1934, 13, 714; Am. J. Clin. Path., 1935, 5, 504.

<sup>1</sup> This study was aided by a grant from the Procter Fund, Harvard Medical School.

anemia, with achlorhydria, and with a prolonged spontaneous remission of symptoms is reported. Two experiments designed to demonstrate the presence or absence of the antianemic "intrinsic factor" of Castle in the gastric juice of this patient are considered to have given negative results. Control experiments are cited, showing positive results with normal human gastric juice and a negative result in pernicious anemia, all in accord with the observations of Castle and his coworkers. We are unable to offer a completely satisfactory explanation for the absence of anemia, for the presence of the combined cord degeneration, or for the failure of the neurologic process to progress during the past two years. Our observations may be interpreted as in accord with either of two views: (a) that combined cord degeneration may be a disease *sui generis*, or (b) that it is invariably a manifestation of the same basic disorder as that which causes pernicious anemia.

*Renal Insufficiency from Blood Transfusion—Relation to the Urinary State.* By ELMER L. DEGOWIN, W. L. RANDALL, E. D. WARNER and W. K. HALL (introduced by H. M. Korns), Iowa City, Iowa.

Certain individuals developing hemolysis from blood transfusions, from quinine idiosyncrasy, or during the course of blackwater fever, die of renal insufficiency. *In vitro*, hemoglobin precipitates in slightly acid solutions. This is increased in amount in the presence of 1 to 2 per cent NaCl. Transfusions of large amounts of dog hemoglobin into dogs with alkaline urines produce innocuous hemoglobinuria. Transfusions into dogs with acid urines produce nitrogen retention and, often, death in coma 4 to 10 days after transfusion. This phenomenon sometimes occurs after the first transfusion but may require several. This inconstancy indicates that the variability of electrolyte content of the urine is also a factor.

Histologic examination of the kidneys of dogs dying in coma shows the tubules to be plugged with pigment derived from hemoglobin. The glomeruli are normal, and the tubular epithelium little damaged. Study of human cases dying from transfusion anuria and from quinine hemolysis indicates degrees of nephropathy varying from extensive tubular necrosis with slight pigment precipitation to cases with little tubular damage and much obstruction from pigment. It seems probable that blockage of tubules by precipitation of hemoglobin in acid urine is responsible for some but not all of the cases of renal insufficiency mentioned.

*Syphilitic Nephritis.* By WARDE B. ALLEN (by invitation) and BENJ. M. BAKER, Baltimore, Md.

The literature on the subject will be reviewed briefly and a critical discussion of the validity of the clinical diagnosis of "syphilitic nephritis" will follow. The pathological basis for syphilitic nephritis will be discussed. The clinical course of several patients who combine syphilis and nephritis will be illustrated by lantern slides. Conclusions will be drawn as to the rational treatment of patients who combine the two conditions.

*Effect of Iodine on Heat Production in Simple Goiter.*

By BRUCE WEBSTER and (by invitation) L. M. WRIGHT, New York, N. Y.

Webster and Chesney in 1928, and Webster in 1929, showed that the administration of iodine to rabbits with large simple goiters resulted in a marked but temporary increase in heat production. It was suggested at that time that this might have a bearing on the production of the so-called "Jod Basedow."

Eight patients with large, recently developed simple goiters were subjected to daily determinations of basal metabolism for one week. They were then given potassium iodide in 1 gram doses daily. An average increase of 18 per cent in heat production occurred. The greatest increase occurred on the third and fourth day after the beginning of the administration of iodine. In spite of the continued administration of iodine, the heat production fell gradually to a level at or slightly above the initial control level. Continued administration of iodine produced no further increase. The same amount of iodine given to four control subjects caused no alteration in basal metabolism.

The average metabolism of 120 consecutive clinic cases of simple goiter was —12 per cent. After three to eight weeks of iodine therapy it was +4 per cent.

These results are compatible with our previous animal experiments. Iodine, administered to a patient with hyperplastic simple goiter causes a temporary outpouring of an excessive amount of thyroxin.

*Clinical Investigation of Patients with Long-continued, Low-grade Fever (Habitual Hyperthermia).* By HOBART A. REIMANN, Minneapolis, Minn.

Twelve persons apparently in good health were intensively studied to determine the cause of low-grade fever, in some cases known to have been present for years. Most of them had visited a succession of physicians in a futile attempt to determine the cause of "fever." Most of them were regarded as tuberculous and three were confined in sanatoria but discharged as non-tuberculous. Of the 12, 2 were found to be normal in all respects as far as could be determined except for temperature, which averaged between 99° and 100° F. These were regarded as instances of true habitual hyperthermia since this average temperature fell within the limits of normal temperature when calculated statistically. The remaining 10 were regarded as distinctly neurotic with evidence of hypochondria, emotional instability, vascular lability, and other symptoms characteristic of neurosis. In all 12 persons the temperature level was uninfluenced by amidopyrine and usually depressed by opium in contrast with fever due to infection; the suspension stability of the blood and the leukocyte picture were normal, agglutinins and intradermal tests for typhoid fever, undulant fever and tuberculosis, and the Wassermann reaction were negative. In 2 patients the basal metabolic rate averaged —25 but was increased by thyroid extract without effect on the temperature.

There appear to be at least two groups of persons

whose temperature may be elevated to fever level in the absence of infection or other organic disease. The first group are apparently normal persons whose temperature is normal for them; the second are those individuals regarded as neurotic whose temperature may be above the usually accepted level of 98.6° F.

*Serum Phosphatase in Diseases of the Liver and the Biliary Tract.* By CHARLES A. FLOOD and ETHEL BENEDICT GUTMAN (by invitation) and ALEXANDER B. GUTMAN, New York, N. Y.

Roberts (1933) claimed that the degree of elevation of serum phosphatase activity could be used to distinguish between jaundice due to extrahepatic biliary obstruction and catarrhal (or toxic) jaundice. Subsequent investigators report conflicting results.

Serum phosphatase activity was determined (by Bodansky's method) in 22 proven cases of obstructive and 33 cases of catarrhal or toxic jaundice. In all our cases of obstructive jaundice, the serum phosphatase exceeded 10 Bodansky units per 100 cc., and the ratio serum phosphatase/serum bilirubin exceeded 1.0. In 29 of the 33 cases of catarrhal or toxic jaundice, the phosphatase was less than 10 Bodansky units. These results suggest that serum phosphatase values < 10 Bodansky units make a diagnosis of jaundice due to obstruction of the extrahepatic biliary tract unlikely. These observations correspond with findings recorded by others following experimental ligation of the common duct and experimental hepatitis in the dog.

In a group of 12 cases of cirrhosis of the liver the serum phosphatase varied widely without any obvious relation to the degree of jaundice.

It is difficult to reconcile the low phosphatase values usually obtained in toxic hepatitis with the view that this type of jaundice is fundamentally due to intrahepatic obstruction.

*Effect of Inhalation of Oxygen under Positive Pressure on Pulmonary Congestion and Edema.* By ALVAN L. BARACH and (by invitation) JOHN MARTIN, New York, N. Y.

The exudation of serum through the lung capillaries into the alveolar spaces may be prevented or made to disappear in certain instances by inhaling oxygen (or air) under 5 to 8 mm. Hg pressure. This offers an opposing pressure to the increased pulmonary capillary pressure. The maintenance within the chest of a positive pressure retards the entrance of blood into the right heart, increases the peripheral venous pressure and slows the circulation time. The clearing of the edema as a complication of cardiac failure persists while the pressure is being applied. Clinical and experimental evidence is presented.

*Immunization against Neoplasms: Its Effect on the Nitrogen Metabolism of the Host.* By WILLIAM T. SALTER and (by invitation) ROBERT OSTER, Boston, Mass.

Ten years ago Dodds found that certain rats which resisted inoculation with neoplasms showed a low blood urea concentration after treatment with x-ray. More

recently, Andervont has demonstrated that inoculation of tumors into the tails of mice may confer immunity against subsequent inoculation even though the tails be amputated. It has been found that animals immunized by Andervont's method show the peculiarity in urea metabolism which Dodds described.

The fasting blood urea concentration of normal and tumor-bearing mice is about 30 mgm. per cent even after treatment with x-ray. Immune animals show the same blood urea until irradiated. Thereupon, blood urea drops steadily for three days to about 20 mgm. per cent, and climbs to normal in the course of the next week.

The excretion of nitrogen in the urine shows urea to be consistently about 82 per cent and ammonia about 8 per cent of the total nitrogen. This is true despite an excessive nitrogen excretion the day after x-ray treatment in both normal and immunized animals. The drop in blood urea, therefore, is not due to a failure of the normal urea-producing mechanism.

Studies of tissue urea (muscle, liver) are correlated with blood studies. The results are interesting because they connect immunity to malignant tissue with an effect of x-rays.

*Variations in Arterial Elasticity as Estimated by Measurements of the Velocity of Transmission of the Arterial Pulse Wave.* By J. MURRAY STEELE, New York, N. Y.

In preliminary studies of arterial elasticity as estimated from the rate of propagation of the pulse-wave, the procedure evolved for obtaining comparable data under different sets of conditions, together with a few of the variations observed, was thought worthy of comment.

The hot-wire sphygmograph (Bramwell and Hill) was employed. The procedure consisted merely in establishing for each subject under investigation, the relation between changes in velocity and diastolic pressure in the brachial artery. Various diastolic pressures were obtained by using positive and negative pressure within cuffs about the arm (Hemingway, McSwiney and Allison). In each case the velocity was found to increase regularly with increase in diastolic pressure. Subsequent variations in velocity from this relation either spontaneous or following procedures known to affect the peripheral circulation could then be evaluated even in the event of marked changes in diastolic pressure. Since after death the elasticity of an artery is, furthermore, remarkably constant for several days, relatively large and abrupt changes during life may be taken as evidence of change in tone of the muscular coats.

Viewed in this light the observed variations in velocity suggest that the tone of the larger arteries varies spontaneously somewhat from day to day; that moderate exercise does not at once affect it to any measurable extent, nor does heat applied directly. If sufficient heat is applied to the body to result in reflex or general dilatation of the peripheral arterioles, then the tone of the larger peripheral arteries also decreases. In two patients suffering from hypertension, the spontaneous changes in

tone, as well as those following heat were more marked than in the normal individuals.

*A Case of Carcinoma of the Liver with Prolonged Asymptomatic Hypoglycemia.* By WILLIAM BENNETT BEAN (by invitation), and READ ELLSWORTH, Baltimore, Md.

The case was that of a 49 year old colored man with an adeno-carcinoma (bile duct) involving fully 80 per cent of the liver. He had a fasting blood sugar of 35 to 45 mgm. per cent without any symptoms or signs except once after fasting 27 hours, he had a typical "insulin" reaction (blood sugar 33 mgm. per cent). Sugar tolerance showed somewhat delayed removal of sugar from blood (opposite to islet tumors). There was no mobilization of glucose after adrenalin (moderate vascular response—control showed 50 per cent rise in blood glucose). Levulose tolerance test showed very slight rise in blood sugar. There was never clinical or laboratory evidence of jaundice, but there was retention of 33 per cent of bilirubin after 30 minutes. Bromsulphthalein showed 80 per cent retention in 35 minutes. Other data: Total protein 6.36 grams. A/G = 42/58. Cholesterol 103 mgm. per cent. Urea N, 25 mgm. per cent. Nonprotein nitrogen 40 mgm. per cent. Phenolsulphonphthalein test 25 per cent in 15 minutes, 70 per cent in 2 hours.

*On the Nature of Insulin Resistance.* By SAMUEL SOSKIN and (by invitation) M. DAVID ALLWEISS, and I. ARTHUR MRSKY, Chicago, Ill.

It has been shown previously that the decreased carbohydrate tolerance occurring in toxemia is not due to a lack of insulin consequent to pancreatic damage but, as we have demonstrated, results from an interference with a homeostatic liver mechanism for blood-sugar regulation. Furthermore, a progressively increasing toxic liver damage presents a characteristic cycle of events, as regards carbohydrate tolerance, which is not merely a progression from the normal to the increasingly abnormal.

In the present report it is shown that, under the same conditions of toxemia which lead to a diminished carbohydrate tolerance, there is also a diminution in the effectiveness of administered insulin. This "insulin resistance," in a progressive toxemia, is subject to and must be interpreted in the light of a cycle of events similar to that which occurs in the carbohydrate tolerance. The loss of liver glycogen reserves is an additional complicating factor.

It is concluded that these results are of value in the interpretation, and of practical importance in the treatment, of cases of "insulin resistance" and of certain cases of so-called hyperinsulinism which present themselves clinically.

*Studies on the Nature of Arterial Hypertension.* By MYRON PRINZMETAL and BEN FRIEDMAN (introduced by B. S. Oppenheimer), New York, N. Y.

The vascular hypertonus that causes arterial hypertension is to be explained by one of the following

mechanisms: (1) Increase in vasoconstrictor impulses. (2) Circulating pressor substances. (3) An intrinsic disturbance in the vessels themselves.

The first factor has been ruled out by Prinzmetal and Wilson who found that anesthetization of vasomotor nerves in hypertension does not release the vascular hypertonus.

No increase in pressor substances in hypertensive plasma was found by perfusion of rabbits' ears. Moreover direct cross transfusions of over one-third of the blood volume between hypertensives and normals were performed; simultaneously, to avoid changes in blood volume. Since no significant change in blood pressure occurred in either group, it appears unlikely that circulating pressor substances cause hypertension.

This leaves the third possibility, namely a local disturbance in the vessels themselves, as the cause. Experiments indicate that this disturbance is associated with the inherent tonus of the vessels, which is present in denervated vessels perfused with Tyrode's solution.

These observations do not rule out the presence of a circulating substance, not pressor in itself, which may be the cause of the pressor mechanism locally in the vessels. The possibility of such a substance seems likely, especially in renal hypertension.

*The Origin and Nature of Normal Synovial Fluid.* By MARIAN W. ROPES (by invitation) and WALTER BAUER, Boston, Mass.

Existing data pertaining to the physical and chemical properties of normal synovial fluid are too few to allow one to speak with any degree of certainty concerning either its nature or origin. Therefore, a very complete physical and chemical analysis of normal synovial fluid and arterial blood as obtained from normal cattle has been made.

From these data one learns that normal synovial fluid is a clear, viscid liquid which does not clot. It contains 131 nucleated cells per cubic millimeter. Specific gravity is 1.010, total solids 2 per cent, pH 7.23, freezing point  $-535^{\circ}$ , osmotic pressure against Ringer's solution 150 millimeters of water. Protein concentration is 1.0 gram per 100 cc., of which one-eighth is mucin.

The concentrations of non-electrolytes are approximately equal in fluid and serum. The concentrations of chloride and bicarbonate are greater in fluid than in serum, while those of sodium, potassium, calcium and magnesium are less in fluid. The concentration of phosphate is approximately the same. The distribution ratios agree with those found for dialysates of blood plasma.

Studies reveal that the subsynovial vascular supply to the knee joint is rich, and in many instances the vessels are separated from the joint space by only a few layers of cells.

Thus, the chemical composition and anatomical arrangement are consistent with the theory that synovial fluid, like lymph and edema fluids, is a dialysate of blood plasma, to which is added mucin.

*The Response of Human Myxedema to an Artificial Thyroid Protein.* By J. LERMAN and W. T. SALTER, Boston, Mass.

Six cases of human myxedema have been relieved by an artificial iodine-containing protein synthesized from human di-iodotyrosine peptone. The basal metabolic rate was used as the measure of potency. The rate of response for the artificial protein averaged 2.5 points (per cent of basal metabolic rate) per day as compared with 2.5 points for standard thyroxine polypeptide given in equi-iodine dosage.

The original di-iodotyrosine peptone produced no response in five patients with frank myxedema, in the same iodine dosage. When doses approximately five-fold were used, however, three patients responded satisfactorily. This effect is attributed to a thyroxine-containing impurity, probably thyroglobulin itself, on the basis of the following evidence. When filtered under 50 atmospheres of pressure through "cellophane" membranes, the original peptone failed in five-fold the standard iodine dosage to relieve three patients. Nevertheless, the artificial protein synthesized from this filtered peptone produced a standard response with standard iodine doses in two patients with myxedema.

The artificial protein ("plastein") behaved like natural thyroglobulin. It contained a thyroxine-like fraction which varied from 12 to 35 per cent of the total iodine. Its metabolic activity was much greater than that indicated by its thyroxine-like content. The latter itself, when isolated by drastic hydrolysis, relieved myxedema.

*The Effect of Pregnancy and of Female Sex Hormones in Modifying the Course of Syphilis in Experimental Animals.* By JAROLD E. KEMP (introduced by Joseph E. Moore), Chicago, Ill.

In 1921 Brown and Pearce reported appreciable alteration in the course of early syphilis in a group of rabbits bred and inoculated simultaneously. This has since been used as experimental confirmation of the clinical assumption that the modified course of syphilis in women is due to pregnancy. The present study was undertaken to confirm Brown's findings and, on the assumption that they were correct, to determine whether or not the hormones found in the urine of pregnant women were the responsible agents.

A group of female animals was inoculated and bred simultaneously and rebred until they had experienced three pregnancies. Another group of twelve males and twelve females was treated daily with pooled pregnancy urine of high estrogenic titer for a period equalling the total duration of pregnancy in the preceding group. While there was marked alteration in the course of the infection in the pregnant group, no appreciable change in the treated groups was noted. The reasons for the difference between the behavior of these animals and those of Fraser which showed marked alteration in the course of the syphilitic infection after treatment with an estrogenic substance of lower titer and the modified course of syphilis in nonpregnant females are discussed.

*The Role of the Pineal Gland in Growth and Development.* By L. G. ROWNTREE, and (by invitation) J. H. CLARK, ARTHUR STEINBERG, N. H. EINHORN and A. M. HANSON, Philadelphia, Pa.

Pineal extract (Hanson) has been administered intraperitoneally daily to parent rats, and the effects have been followed through six generations. The result was a marked retardation in the rate of growth and marked acceleration in the rate of development—precocious "dwarfism" with relative macrogenitalism. The effects of pinealectomy were studied in four succeeding generations of parents. The results to date have been inconsistent.

*The Reticulocytosis of Fetal and Nursling Rats. Preliminary Report of a Method of Investigation of Some Factors Influencing Reticulocyte Maturation.*<sup>1</sup> By THOMAS FITZ-HUGH, JR. and (by invitation) ADOLPH J. CRESKOFF and HELEN B. TAYLOR, Philadelphia, Pa.

Five living rat fetuses of different ages were extracted<sup>2</sup> from five mothers for blood study. The daily levels, from birth to twentieth day, were determined, of reticulocytes, erythrocytes and hemoglobin, of forty rats from fourteen litters.<sup>3</sup> A virtually 100 per cent reticulocytosis was found throughout fetal life and for twenty-four hours after birth (suggesting fetal reticulocyte maturation-arrest). Then the reticulocyte curve descended to 25 per cent during the next twenty days. Concomitantly from birth, there was a rise of erythrocytes, indicating peripheral reticulocyte maturation and suggesting "release of inhibition" or postnatal acquisition of a stimulant to complete maturation.

We attempted to "stimulate" (with parenteral liver extract) reticulocyte maturation during late fetal and nursling life, by injecting nine individual fetuses<sup>4</sup> *in utero* (two operated mothers) and by daily postnatal injections to fourteen nurslings (five litters). No significant change from our normal reticulocyte curve was obtained.

Conclusions: 1. The method offers technical advantages for study of reticulocyte maturation *in vivo*. 2. Evidence suggesting peripheral reticulocyte maturation is presented. 3. The virtually 100 per cent reticulocytosis of fetal and new-born rats suggests fetal inhibition, or lack, of a factor normally affecting the final stage of reticulocyte maturation. 4. Liver extract failed to stimulate reticulocyte maturation under these conditions.

*The Therapeutic Effect of Venesection in Polycythemia.* By D. J. STEPHENS and (by invitation) NOLAN KALTREIDER, Rochester, N. Y.

The effect of venesection has been studied in five patients with polycythemia. Observations of the red

<sup>1</sup> Aided by a grant from Mrs. S. duPont Ford.

<sup>2</sup> Nicholas, J. S., *Anat. Rec.*, 1925, 31, 385.

<sup>3</sup> Fitz-Hugh, T., Jr., Robson, G. M., and Drabkin, D. L., *J. Biol. Chem.*, 1933, 103, 617.

<sup>4</sup> Corey, E. L., *Physiol. Zool.*, 1930, 3, No. 3.

blood cell count, hemoglobin, hematocrit, reticulocytes, and, in some instances, the blood volume, viscosity and circulation time (arm to tongue) were made before, during and after ten periods of bleeding. The removal of from 1000 to 3000 cc. of blood within a few days, by means of repeated phlebotomies of from 200 to 600 cc. each, resulted in hematological and clinical remissions varying in duration from a few months to two years. Except for two instances in which a maximum reticulocytosis of 6.3 and 4.3 per cent, respectively, was observed, the reticulocytes remained within the normal range during and after the venesection periods.

The therapeutic effect of venesection compares favorably with that of other measures which have been used in the treatment of polycythemia. Reduction of the blood volume, hematocrit and viscosity by means of venesection has the theoretical advantage of removal from the organism of iron and other potential blood building materials, which are in large part retained and stored by patients in whom the therapeutic effect is produced by means of the hemolytic action of phenylhydrazine.

*Further Experiences with Sternal Puncture in the Study of Bone Marrow Changes in Various Hematological Conditions.* By G. O. BROWN and (by invitation) W. F. HOLMES and J. J. FURLONG, St. Louis, Mo.

In 1933, Holmes and Brown<sup>1</sup> reported the use of sternal puncture for the clinical investigation of bone marrow. Fifty cases were included in the first report—this being the first extensive study of marrow specimens obtained by needle puncture under local anesthesia, reported in American literature. Further experience with the method has been secured in the intervening period.

Marrow specimens so obtained are well adapted for study of comparative morphology of marrow cells and peripheral blood cells. It can be shown that contamination with peripheral blood is usually not great. Variations in peripheral blood in general are closely reflected in changes in more immature cell forms in the marrow specimens. Repeated punctures are practicable and offer a means of studying the effects of treatment in hematological disturbances.

In untreated pernicious anemia, the megaloblastic marrow changes are readily demonstrated. A shift to more normal erythrocytic forms occurs under liver therapy.

Therapeutic malaria has been studied during various phases of the disease with definite marrow changes noted.

Characteristic marrow pictures have also been found in hemolytic icterus, acute agranulocytosis, leukemias, aplastic anemia, eosinophilia, lymphocytosis, and acute and chronic infections.

*The Excretion of Xylose, Sodium Benzoate, and Urea in Pernicious Anemia in Relation to Maintenance Dosage of Liver Extract.* By L. G. ZERFAS and (by invitation) O. M. HELMER and P. J. FOUTS, Indianapolis, Ind.

As a possible test of absorption twenty-five grams of

xylose (a non-metabolized sugar) was administered to each of forty-seven patients having pernicious anemia. In addition Quick's sodium benzoate liver function test and urea clearances were run on these patients. It was found that, except for several individual exceptions, the xylose excretion and sodium benzoate excretion tended to follow the urea clearance. The urea clearance varied from 23 to 150 per cent of normal. There was a distinctly lower average urea clearance in the patients tested when the red blood cell count was below 4.5 million than in those tested when the red blood cell count was normal. Of the patients examined before and after liver therapy, the four patients known to require liver extract by injection had no increase in urea clearance following liver therapy. All the patients responding satisfactorily to liver extract administered by mouth and who had low urea clearances when in relapse have subsequently shown definite increases in urea clearance. The average urea clearance of the patients requiring liver extract by injection was distinctly lower than that of those responding to oral therapy. No patient having a urea clearance below 50 per cent of normal after maintaining the red blood cell count at normal levels for several months was able to keep the blood at normal levels without injections of liver extract.

*On the Fate of Reticulocytes in the Blood Stream.* By RAPHAEL ISAACS, Ann Arbor, Mich.

The blood, during reticulocyte remissions in patients with pernicious anemia, was studied to note the numerical behavior of the reticulocytes and the next older stage, the granule red blood cells. The absence of a proportional increase in granule red blood cells after an increase in the number of reticulocytes, is interpreted as indicating that reticulated red blood cells do not mature in the peripheral circulation, but are removed as such. There is evidence that they are removed by the phagocytic cells in the spleen.

*Chlorosis.* By ARTHUR J. PATEK, JR. (introduced by Dr. Laurence B. Ellis), Boston, Mass.

Chlorosis is described today as a disease of unknown etiology, which has disappeared mysteriously. Because of the supposed rarity of this disease, four typical severe cases are presented. Since the hypochromic anemia responded readily to the administration of iron, study of causes for iron deficiency in these patients was made.

It is apparent from the analysis that the demand for iron made by growth, loss of iron by menstrual and other blood loss, and insufficient intake of iron are the three important factors in the production of chlorosis. The mothers of two of the four cases had been anemic. It is likely that these patients inherited a meager store of iron at birth.

*Conclusions.* (1) Chlorosis has not disappeared. (2) Chlorosis is the exaggeration of a normal tendency towards anemia in adolescent girls, created by the increased demand for iron made by growth, by menstrual or other blood loss, and by a diet insufficient in iron-containing foods.

<sup>1</sup> Holmes, W. F. and G. O. Brown, Proc. Soc. Exper. Biol. and Med., 1933, 30, 1305.



*The Effect of a Deficient Diet on the Blood Picture of the Guinea Pig.* By THEODORE G. KLUMPP and (by invitation) EMIR A. GAW, New Haven, Conn.

With Castle's fundamental experiments as a basis, attempts were made to produce in the guinea pig a deficiency state similar to human pernicious anemia. As an approach to this problem the effect on the blood picture of a diet which might be deficient in the extrinsic factor of Castle was studied. The following diet was employed: Salted soda crackers buttered with a mixture of vitamin free casein, reduced iron, white flour and water; lettuce or cabbage; crystalline vitamin B<sub>1</sub>; cod liver oil or carotene and viosterol; and cysteine hydrochloride.

Despite a variable weight loss many of the animals lived longer than two months and some indefinitely. When cysteine hydrochloride was withdrawn, alopecia and ulcers of the skin developed. No consistent blood changes were noted. In some of the animals the appearance of prolonged low reticulocyte levels was observed but abrupt increases occurred spontaneously and after injections of liver extracts with equal frequency. Parenteral liver extract failed to influence, in any particular, either the weight or blood picture of the guinea pigs.

*The Preservation of Virulent Treponema pallidum and Treponema pertense in the Frozen State.* By THOMAS B. TURNER, New York, N. Y.

Hitherto it has not been possible regularly to maintain the spirochetes of yaws or syphilis in a virulent state outside an animal or human host for longer than a few days. Except in rare instances these organisms lose pathogenicity for laboratory animals when grown on artificial media. When, however, *T. pallidum* and *T. pertense* are frozen at temperatures approximating -78° C. and maintained at this temperature they retain normal morphology and motility, and their virulence for rabbits is essentially unchanged after at least four months.

Infectious material from rabbits was stored in a thermos jug containing dry ice (solid CO<sub>2</sub>) and 95 per cent alcohol. Eight specimens of syphilis virus (Nichols strain) and 6 specimens of yaws virus have been tested before freezing and at intervals of 2 weeks, 1 month, 2 months, and 4 months after freezing. After these intervals the appearance of the organisms was unchanged and upon inoculation of rabbits the incubation period and the character of the initial lesion did not vary significantly from that produced by the same lot of material before freezing. Material from 7 additional strains of yaws spirochetes and lymph node material from 5 different strains of syphilis virus have been tested after 2 months with similar results. With one exception, syphilis spirochetes were not pathogenic for rabbits after desiccation.

*Observations on Plasma Fibrinogen Responses.* By THOMAS HALE HAM (introduced by George R. Minot), Boston, Mass.

The blood plasma fibrinogen in 30 normal individuals

was found to be within the limits of 180 to 330 mgm. per 100 cc. of plasma; the usual values ranged from 220 to 270 mgm. Repeated fibrinogen determinations were done on one of the normal subjects, and remained remarkably constant over a period of 15 months. In 6 cases of pernicious anemia and one of scurvy, all with severe anemia, the plasma fibrinogen varied from 168 to 272 mgm.

After 5 days of high protein feeding, two normal subjects showed a slight increase in fibrinogen, reaching a maximum after a week, in one subject, and after 12 days in the other. An average fibrinogen level of 180 mgm. was observed in a pernicious anemia patient, following a prolonged low protein diet; when fed a diet rich in liver, over a period of 10 weeks, the fibrinogen increased gradually to an average level of 270 mgm.

Fibrinogen responses were studied in: (1) infectious, degenerative and neoplastic diseases; (2) following the intravenous injection of typhoid vaccine; and (3) in hyperthermia induced by high environmental temperature. The elevation of plasma fibrinogen was found to be one of the most frequent, non-specific responses of the body to noxious stimuli; it was similar in nature to the temperature and white blood cell responses, but was independent from them. Two to three days after the injection of a single, large dose of typhoid vaccine in normal subjects, the fibrinogen rose to a maximum, and required from 6 to 10 days to return to its previous value. Artificially induced hyperthermia produced only a slight, temporary fibrinogen rise. Prolonged elevation of the fibrinogen was associated with chronic infection and, in 2 cases, with neoplasm.

The fibrinogen failed to rise in 3 cases of overwhelming infection; autopsy revealed severe liver damage in 2 of these cases, but only slight damage in one. Fibrinogen failed to rise in one case of toxic hepatitis, showing severe liver necrosis at postmortem.

*The Advantage to the Diabetic of Protein as a Source of Glucose.* By JEROME W. CONN (by invitation) and L. H. NEWBURGH, Ann Arbor, Mich.

The capacity of a diabetic to dispose of glucose is measured in terms of the calculated total glucose released from his diet in 24 hours. Little consideration has been given to the rate at which this release occurs. These experiments show that rate is as important a factor as total yield.

Blood sugar curves following ingestion of glucose were compared with those following ingestion of enough protein to yield equivalent amounts of glucose. Normals and diabetics were studied. Blood urea nitrogen determinations were used to indicate rate of metabolism of protein.

The slowly rising blood urea nitrogen after ingestion of protein indicates the slow rate of deamination of amino acids in the liver. The glucose derived in this process is thus made available at a relatively slow rate. The inability of a diabetic to dispose of large quantities of glucose is partially compensated if the glucose is pro-

sented for utilization slowly and evenly. In thirteen diabetics studied the average maximum increase in blood sugar after ingestion of carbohydrates was 160 mgm. per 100 cc. and was invariably attended with significant glycosuria. When an equivalent amount of glucose was derived from protein the average maximum increase was 37 mgm. per 100 cc. and produced no glycosuria.

*Experimental Production of Macrocytic Anemia in Pregnancy.* By KATHARINE O'SHEA ELSOM (introduced by T. Grier Miller), Philadelphia, Pa.

A macrocytic anemia was produced in eight pregnant women who, from the fourth month until delivery, were given an experimental diet deficient in the vitamin B complex. The diet was calculated to contain approximately one-half of the theoretical vitamin B requirement, to be adequate in all other vitamins, in calories and in protein. Additional iron was given as ferrous sulphate, and vitamins A and D, as haliver oil and viosterol. Control patients who received a completely adequate diet plus the supplements mentioned, did not develop anemia. The anemia was characterized by a slight fall in red cells and hemoglobin, slight increase in color index, marked increase in mean corpuscular volume, reticulocytosis, macrocytosis and the appearance of poikilocytes, polychromatic cells and immature white cells in the stained blood. The anemia responded to brewer's yeast and to Liver Extract No. 343 intramuscularly.

Gastric acid was variable in the anemic patients. Tongue changes were also variable, some patients showing no change, others showing marked glossitis. All complained of gastro-intestinal distress. Numbness and paresthesias of the lower extremities occurred in all the patients on the deficient diet and in some was accompanied by temporary loss of vibratory sense. These symptoms and physical signs, not present in the control patients, disappeared promptly after administration of brewer's yeast or liver extract.

*The Use of Ferrous Gluconate in Hypochromic Anemia.*

By PAUL REZNIKOFF and (by invitation) WALTER F. GOEBEL, New York, N. Y.

To obtain a ferrous compound which is soluble and does not coagulate proteins *in vitro* or cause necrosis when injected, ferrous gluconate was made. Ferrous gluconate prepared aerobically was given by mouth or by injection to 16 milk-fed rats derived from milk-fed mothers, so that they received 1 mgm. of iron daily. Seven control animals were also studied. Reticulocytes rose promptly to a peak within five days. A fairly high hemoglobin level occurred in the animals receiving the gluconate orally in three weeks; in the injected animals in two weeks.

Eight patients suffering from hypochromic anemia have been treated with ferrous gluconate to date, three by injection and five by oral administration. Prompt reticulocyte rises and adequate hemoglobin responses have ensued. No unpleasant systemic effects were seen and practically

no local reactions to the injections occurred even when a quantity containing 25 mgm. of iron was given daily.

Ferrous gluconate is best prepared by adding one equivalent of ferrous sulfate to an aqueous solution of barium gluconate in an atmosphere of nitrogen and then concentrating and filtering the crystalline derivative from the mother liquor.

*The Rôle of Lipids in Immune Reactions.* By FRANK L. HORSFALL, JR., and K. GOODNER (introduced by Thomas M. Rivers), New York, N. Y.

Specific agglutination and precipitation cannot be obtained with horse and rabbit antipneumococcus sera from which the lipids have been removed. This extraction, however, does not affect the capacity of the antibody to unite with the specific antigen. The full reactive properties of antipneumococcus horse serum return upon the addition of small amounts of lecithin. For antipneumococcus rabbit serum cephalin is required for restoration.

These phosphatides, which appear to be *essential* for the *in vitro* demonstration of immune reactions, must be carefully distinguished from the large amount of heterogeneous and *non-essential* lipid found in immune aggregates. The latter, for example, may constitute from 5 to 80 per cent of an immune precipitate, and appears, for the most part, to have been non-specifically absorbed. The single exception to this lack of specificity is that with immune rabbit serum, lecithin is selectively absorbed, whereas horse serum immune aggregates fix cephalin. Thus the non-essential phosphatides occupy a position paradoxical to those of the essential.

It seems not unlikely that, in addition to the important rôle of lipids in the *in vitro* demonstration of immune reactions, they exert a profound influence in the capacity of an animal to utilize antibodies.

*Multiple Cases of Pneumococcal Infections in Families with the Demonstration of the Development of Antibodies in Contact Carriers.* By R. CARMICHAEL TILGHMAN (by invitation) and MAXWELL FINLAND, Boston, Mass.

The evidence obtained from a study of multiple cases of pneumococcal infections in contact groups indicated that they resulted from exposure to an antecedent case. With the exception of two instances, all the pneumococcal infections were observed in families. Twenty-three groups included fifty-four individuals ill of pneumococcal infections of homologous types. The majority of the contact infections occurred in less than fourteen days after exposure, and the clinical manifestations within the groups suggested a similar severity.

Detailed bacteriologic studies made on the entire family and contacts of five of the groups demonstrated a high incidence of carriers of the pneumococcus responsible for the infection in the relatives. Immunologic data obtained from a study of the blood of the carriers and non-carriers of the disease-producing pneumococci showed that specific antibodies may develop in those individuals who are carriers but who do not become ill with the in-



fection. One instance was encountered in which an individual, who did not possess antibodies against pneumococcus Type V, developed specific agglutinins and mouse protective antibodies after he became a carrier of the Type V pneumococcus without any evidence of infection. In another instance, pneumococcus Type XXII agglutinins in low titer appeared during convalescence from Type V pneumonia, and later the Type XXII was cultured from the patient's throat.

*The Excretion of Electrolytes in the Absence of the Colonic Absorption of Intestinal Fluids.* By STUART WELCH (by invitation) and E. G. WAKEFIELD, Rochester, Minn.

We have observed the extent of loss of fluids and electrolytes among patients who had been subjected to ileostomy and those who had disease characterized by a chronic diarrheal state. Among those who had an ileac stoma, the excretion of base through the loss of intestinal fluid was apparent as 90 per cent of the sodium was excreted in the ileac dejecta. However, there was a compensatory decrease of the sodium in the urine to 10 per cent of the total amount of sodium excreted, whereas the excretion of chloride in the ileac dejecta was 30 per cent of the total chloride excreted.

In chronic diarrheal states, an increased loss of fluids and electrolytes in the feces similarly produces a decrease in the amount of fixed base in the urine. The excretion of chloride in the feces, as in the cases in which the patients have been subjected to ileostomy, amounts to 30 to 60 per cent of the total excretion of chloride.

Excessive loss of ileac excreta may produce clinical symptoms comparable in some ways to those observed in states of suprarenal insufficiency; and just as in suprarenal insufficiency, these symptoms also may be relieved by replacing fluid and electrolytes.

*The Heart Fifteen to Twenty Years after Severe Diphtheria. A Follow-Up Study of 100 Cases.* By WILLIAM PAUL THOMPSON and SHERMAN E. GOLDEN (by invitation), and PAUL D. WHITE, Boston, Mass.

In 1926, one hundred individuals were examined five to ten years after severe diphtheria. No evidence of heart disease was found, although a recent review of the electrocardiograms taken then has revealed four cases with a duration of the Q.R.S. complexes of 0.11 second and prominent S waves in Lead II, previously considered normal but today open to some question. These four cases may or may not have had these rather wide Q.R.S. waves before their diphtheria; we have encountered similar complexes in the routine examination of occasional individuals with normal hearts.

Eighty-three of the original one hundred cases have now been re-examined, and two more are known to be dead, one of rheumatic heart disease and one of pneumonia. Two of the eighty-three cases followed up now have arterial hypertension but normal sized hearts and two have rheumatic heart disease. Electrocardiograms in the eighty-three cases are essentially unchanged after

the additional years. The four with questionably abnormal Q.R.S. complexes now have almost identical electrocardiograms. There is no case with an abnormally long P-R interval, although in three this interval measures 0.20 second (the same as ten years ago in one case, while the other two have increased to this length from the 0.15 and 0.16 seconds they showed before).

An additional seventeen cases have been examined, so that the present series actually examined numbers one hundred. These likewise had severe diphtheria fifteen to twenty years ago. One has a mild hypertension and another rheumatic heart disease, but electrocardiographic abnormalities were not found.

This follow-up series of cases has revealed then, 15 to 20 years after severe diphtheria, no evidence of heart disease that can be definitely attributed to the diphtheria.

*The Chloride Content of the Cerebrospinal Fluid in Various Diseases and Its Relationship to the Chloride Content of the Serum.* By H. HOUSTON MERRITT and FRANK FREMONT-SMITH, Boston, Mass.

The chloride content of the cerebrospinal fluid of 2,953 patients with various diseases has been determined. In 268 of these patients simultaneous determinations of the chloride content of the serum were made. The results can be summarized as follows:

1. The chloride content of the cerebrospinal fluid is normal or very near to the normal limits in patients with diseases not accompanied by fever, vomiting or evidences of an inflammatory reaction in the nervous system.

2. The chloride content of the cerebrospinal fluid is reduced in patients with diseases accompanied by fever, vomiting or evidences of an inflammatory reaction in the nervous system. Very low values may be found occasionally in patients with any of these diseases, but they are most frequent in patients with meningitis.

3. The chloride content of the cerebrospinal fluid may be reduced, or it may be greatly increased in patients with uremia.

4. The normal ratio of the chloride content of the cerebrospinal fluid to that of the serum is as 1.2 is to 1. This ratio is also usually found in patients with an abnormal chloride content in the cerebrospinal fluid.

The amount of chlorides in the cerebrospinal fluid is dependent upon the concentration of the chlorides in the serum. Changes in the chloride content of the cerebrospinal fluid are chiefly a reflection of changes in the serum. Other factors such as the protein content and acidity of the fluid play only a minor rôle.

*Vitamin C and Infection.* By JAMES M. FAULKNER and (by invitation) F. H. L. TAYLOR, Boston, Mass.

Titration of reduced ascorbic acid in blood serum and plasma with 2,6 dichlorophenolindophenol solution has been utilized in the study of the effect of infection on the vitamin C metabolism. The method used employs certain modifications of previously described techniques which, it is believed, increase its accuracy.

With this technique estimations of the level of ascorbic

tamic acid in the blood of 33 normal individuals varied from 0.83 to 2.43 mgm. per 100 cc. In 13 adult patients with clinical scurvy the values ranged from 0.24 to 0.63 with an average value of 0.47. In a study of 50 patients with various infectious diseases who were previously known to have been on adequate diets as regards vitamin C, it was found that the blood levels of cevitamic acid were frequently reduced to levels seen in scurvy. In such patients the urinary output of vitamin C was low and did not rise after the oral administration of a single large dose (1 gram) of the pure substance although the blood level regularly showed a slight rise lasting over several hours. The same response has been noted in patients with scurvy without infection. In the presence of chronic active infection, such as advanced pulmonary tuberculosis, large daily doses of vitamin C (between 300 and 500 mgm.) were required to bring the blood value up to the normal level and to maintain it there.

The findings are confirmatory of previous observations by others and ourselves that the requirements of the body for vitamin C during infection may be greatly increased.

*A Peptide of Thyroxine with Greater Calorigenic Activity Than Desiccated Thyroid: A Consideration of Methods of Assay.* By W. O. THOMPSON and (by invitation) S. B. NADLER, P. K. THOMPSON, S. G. TAYLOR, III, and L. F. N. DICKIE, Chicago, Ill.

In 14 of 17 patients with myxedema the oral administration of a peptide of thyroxine produced about 30 per cent more calorogenic activity per milligram of iodine than desiccated hog thyroid. In 12 of 16 patients it was about one-third more active than thyroxine when both were given by the subcutaneous route.

Since the accuracy of deductions depends upon the accuracy of methods of assay, a large series of observations was carried out to determine the best method in man, with the following results:

(1) A given amount of thyroid produced about one-third more activity when administered in divided doses over a period of 13 days than when given in a single large dose. However, the increased activity of the peptide was evident by both methods. Data now being collected by a third method (maintenance dose) also suggest that the effect of the peptide is greater.

(2) Erroneous conclusions may be drawn if:

(a) The amount and duration of the increase in metabolism are not observed as well as the rate at which the increase occurs.

(b) The doses compared do not contain the same amount of iodine and the comparison is not made in the same patient.

These findings have an important bearing on the hypothesis that the activity of thyroxine is enhanced by its natural combination and that the activity of desiccated thyroid is not proportional to its total iodine content.

*Acoustical Properties of Stethoscopes of Various Types.*

By FRANKLIN D. JOHNSTON, Ann Arbor, Mich.

To obtain information regarding the sound transmis-

sion properties of commonly used stethoscopes, a number of end-pieces both of the bell and Bowles type have been tested. These studies were made while the various units were actually transmitting sounds derived from the body, or under conditions acoustically similar. The results indicate that most of the bell type end-pieces tested behave in a similar manner for both low and high pitched sounds. The selectivity for high pitched sounds displayed by the Bowles type unit was found to be due entirely to the presence of the diaphragm, since its removal destroyed this property.

Using fundamental acoustic principles concerning the transmission of sound energy from a dense into a rare medium it can be shown that an important function of a stethoscope is to increase materially the very small per cent of energy usually transmitted across the chest wall. The application of these ideas to stethoscope end-piece design is discussed. Studies relating to air conduction through tubes of different types are now in progress.

*The Effects of Mecholine on the Frog's Heart.* By A. E. COHN and A. G. MACLEOD, New York, N. Y.

The influence of pharmacological agents and pathological changes upon the fundamental properties of heart muscle still requires study. In examining the effects of such agents as mecholine and the pure glucosides of the strophanthin group certain important phenomena were observed. In this communication the effects of mecholine only will be described. The drug was given intravenously to large frogs and action currents recorded with cephalad-caudad and direct leads from the surface of the heart. The duration of the secondary deflection of both auricle and ventricle was greatly shortened; the portion of the curve which corresponds to the R-T segment of the electrocardiogram was consequently short. *Pari passu* the refractory period shortened. Presumably because of the last mentioned effect ectopic rhythms often developed simulating fibrillation and flutter. The phenomena were transient and complete recovery occurred within a short time. All the effects of the drug were abolished by atropine. Atropine by itself produced no detectable effect. The relation of these phenomena to the intrinsic and extrinsic nervous mechanisms of the heart is still being investigated.

*Studies of the Circulation in Spontaneous Myxedema.*

By HAROLD J. STEWART and (by invitation) JOHN E. DEITRICK and NORMAN F. CRANE, New York, N. Y.

This paper presents a summary of the data obtained in a study of the circulatory functions of the four patients suffering from typical spontaneous myxedema. Numerous observers have studied individual functions of the heart and circulation in myxedema, but we have attempted to gain as complete a picture of the circulation as possible by the use of various methods applied to the same individual. We have measured the cardiac output (acetylene method, 3 samples of gas being taken), arm to tongue circulation time (dechlorin), venous pressure (direct method), cardiac size (2 meter x-rays),



### Serum electrolyte partitions in lymphogranuloma inguinale

Complete serum electrolyte partitions were carried out in 18 cases of lymphogranuloma inguinale (Table II). In 10 instances the sum of the determined acid equivalents exceeded the total base, i.e. B—A appeared to be negative. Every case of lymphogranuloma inguinale in this series in which the euglobulin fraction was increased to 1.4 per cent or more exhibited this apparent discrepancy in acid-base equivalence.<sup>4</sup> There was, in fact, a fairly well defined tendency for the discrepancy in acid-base balance to be more marked as the euglobulin content increased, a relationship approximately linear. This trend is illustrated in a scatter diagram in which the euglobulin content of sera containing normal and increased amounts of euglobulin is plotted against total base minus total determined acids (Figure 1).

Consideration of the individual constituents of the acid-base balance in these cases of lymphogranuloma inguinale (Table II) reveals little of significance with the exception of protein where this was elevated. In several instances, the sodium content of the serum fell to 136 m.eq. per liter or below, for reasons wholly obscure. While the fall in sodium may contribute to the apparent excess of total determined acids over total base, in most cases the discrepancy in acid-base equivalence was associated with sodium values within normal limits. In several cases, the serum albumin was definitely decreased (Table I). For the most part, the decrease was associated with proteinuria (1), notably in Cases E. A. and C. K.

Wassermann reaction of the blood was positive in 12 of our 35 cases, the Dmelcos test was positive in 6 patients. Evidence of tuberculosis was noted in only 2 cases, but no systematic investigation in this direction was carried out. Parenteral administration of arsenic, bismuth and gold compounds may affect the level of serum proteins, it is said, but hyperglobulinemia was observed in 24 patients in this series before such treatment was begun.

<sup>4</sup> The term "euglobulin" as used in this paper applies to the protein complex precipitated from serum by a concentration of 1M sodium sulfate, according to Howe's procedure. It is appreciated that the chemical identity of the euglobulin fraction is not established, that the salting-out process does not allow of sharp fractionation and that separation of the several protein fractions probably causes reversible changes in the several protein component systems.

TABLE III  
Multiple myeloma: Serum electrolyte partitions; serum protein fractions in 6 cases

Case	Sex	Date	Cl	HCO <sub>3</sub>	Protein	CO <sub>2</sub>	Na	K	Ca	Total base	Total acid	B-A	Non-protein nitrogen	Total protein	Albumin	Globulin	Euglobulin	Remarks
			m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	m. eq. per liter	mgm. per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	
J. B.	♂	February 1, 1935	98.5	28.9	21.5	135.0	4.5	4.7	10.8	151.1	151.1	-1.0	20	10.0	2.2	0.8	4.1	Diagnosis proven at autopsy
		February 13, 1935	90.0	27.0	23.0	133.8	4.2	4.7	11.7	152.9	152.9	-0.2	30	10.3	4.2	7.1	4.0	
		April 21, 1935	97.6	22.5	20.0	127.8	3.7	4.1	12.0	141.0	141.0	-3.1	45	9.2	3.1	6.1	2.8	
		February 10, 1936	97.6	22.5	20.0	127.8	3.7	4.1	12.0	141.0	141.0	-3.1	45	9.2	3.1	6.1	2.3	
A. T.	♂	May 11, 1936	99.5	28.1	19.5	133.4	4.8	4.9	11.5	149.1	149.1	-4.0	37	8.6	3.8	4.8	1.8	Bone marrow biopsy; plasma cell myeloma
P. F.	♂	October 22, 1935	112.8*	20.8	18.7	132.4*	5.1	8.5	107.3	103.1	+5.2	125	9.0	2.0		7.0		Mg determined: 1.3 m. eq. per liter. Sulfate not determined. Chloride findings summarized elsewhere (239), Table III, Case 41
R. L.	♂	November 8, 1935	106.2	21.3	13.1	111.5	5.0	0.8	135.3	111.7	+10.0	83	5.5	4.1		1.0	0.2	Diagnosis proven at autopsy
		February 17, 1936	111.0	18.9	11.0	137.5	5.9	0.7	137.5	138.5	+0.0	80	5.5	3.0		1.9		Sulfate determined: 4.0 m. eq. per liter
		February 21, 1936	111.0	18.9	11.0	137.5	5.9	0.7	137.5	138.5	+0.0	80	5.5	3.0		1.9		Diagnosis proven at autopsy
A. C.	♂	March 1, 1931	91.1	24.3	10.3	121.1	3.2	0.5	142.8	136.7	+6.1	65	9.9					Chloride findings summarized elsewhere (239), Table III, Case 41
O. D.	♂	April 22, 1930	101.5	23.1	10.1	130.1	4.5	7.1	152.7	147.0	+5.7	73	7.7	5.1		2.3	0.4	Sulfate determined: 4.8 m. eq. per liter. Diagnosis based upon typical x-ray findings and clinical course; hence Jones proteinuria

\* Contamination with NaCl

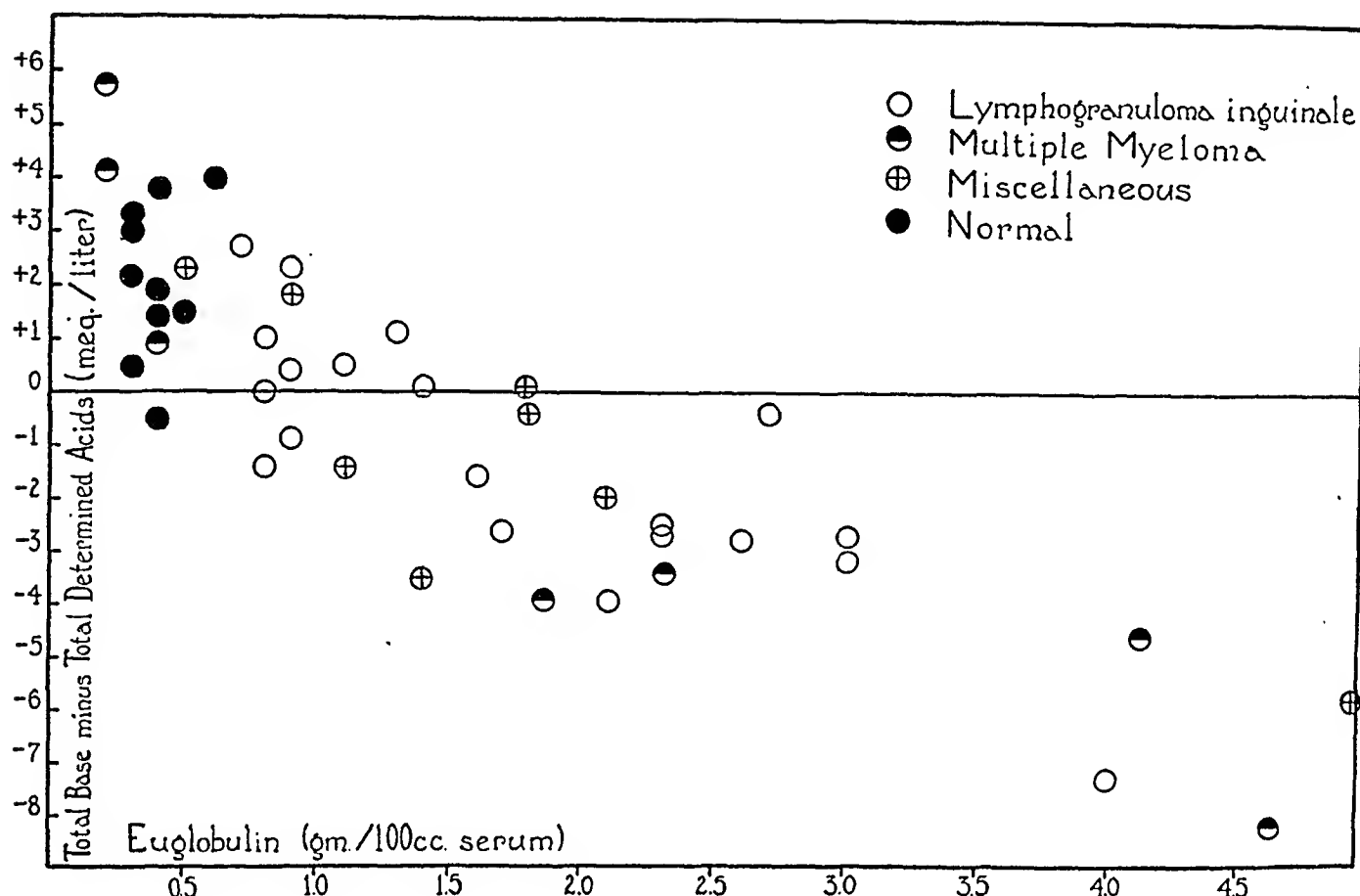


FIG. 1. SCATTER DIAGRAM IN WHICH  $B - A$  IN MILLI-EQUIVALENTS PER LITER IS PLOTTED AGAINST EUGLOBULIN CONTENT IN GRAMS PER 100 CC. SERUM IN 10 NORMAL SUBJECTS, 18 CASES OF LYMPHOGRANULOMA INGUINALE, 4 CASES OF MULTIPLE MYELOMA AND 8 MISCELLANEOUS CASES WITH HYPERGLOBULINEMIA.

In the cases of multiple myeloma with nitrogen retention in which sulfates were determined, the values obtained for sulfates have been added to the sum of acid-equivalents.

The figure shows a trend in the direction of increasing excess of total determined acids over total base in bloods containing increasing amounts of euglobulin.

similar increase in euglobulin may occur in malaria (27), filariasis (28), syphilis (29, 30), tuberculosis (31, 32), rheumatoid arthritis (33, 34) and other chronic infections; but the incidence of patients presenting hyperproteinemia appears to be considerably lower, and values over 9.0 per cent are exceptional. In multiple myeloma (35), as is well known, the concentration of proteins in the serum may reach extraordinarily high levels.

It is apparent that the hyperproteinemia occurring in lymphogranuloma inguinale is not the result of a decrease in the volume of circulating fluids since this would not account for the alteration in A:G ratio. Moreover, hematocrit determinations of blood cell volume, carried out in 3 cases, gave normal values. None of the patients in this series presented the clinical picture of dehydration. It is probable that the hyperglobuline-

mia observed in lymphogranuloma inguinale, like that noted in other chronic suppurative infections, reflects the response of the organism to certain types of infection.

Hyperproteinemia is neither as constant nor as specific a manifestation of lymphogranuloma inguinale as is the Frei reaction. It is apparent, however, that the possibility of lymphogranuloma inguinale should be considered in cases presenting unexplained hyperproteinemia, particularly in negroes, and that this possibility should be ruled out by means of the Frei test.<sup>3</sup>

<sup>3</sup> In a disease as widespread as lymphogranuloma inguinale appears to be in the negro population of the United States (36, 37), it is of course possible that the association with hyperproteinemia may be wholly coincidental, the exciting cause being in reality some co-existing infection such as syphilis, chancroid or tuberculosis. The

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A. T.	58	♂	May 11, 1936	99.5	28.1	19.5	1.7	133.1	4.8	4.9	115.1	102.1	-1.0	37	8.0	3.8	4.3	1.8	Bone marrow biopsy: plasma cell myeloma
P. F.	60	♂	October 29, 1933	113.9 <sup>*</sup>	20.3	18.7	2.8	132.1 <sup>*</sup>	5.1	8.5	107.3	102.1	+5.2	125	9.9	2.0	7.0	7.0	Mg. determined: 1.3 m.eq. per liter. Sulfate not determined. Clinical findings summarized elsewhere (38). Table III, Case 4
B. C.	61	♂	November 8, 1935 February 17, 1936 February 21, 1936	104.2 114.0	21.3 18.0	19.4 11.0	3.8 3.4	141.5 143.3	5.0 5.0	0.8 0.7	155.3 137.3	111.7 118.5	+10.0 +9.0	83 80	5.5 5.0	4.1 3.0	1.0 1.9	0.3	Diagnosis proven at autopsy. Sulfate determined: 4.0 m.eq. per liter
A. C.	47	♂	March 1, 1931	94.1	24.3	16.3	2.0	131.1	3.2	0.5	112.8	130.7	+0.1	65	6.0			0.1	Clinical findings summarized elsewhere (39). Table III, Case 9
O. D.	40	♂	April 22, 1930	101.5	23.1	19.1	3.3	139.1	4.5	7.1	152.7	147.0	+5.7	73	7.7	5.4	2.3	0.1	Sulfate determined: 4.8 m.eq. per liter. Discrepancy based upon typical x-ray findings and clinical course; Rosen Jones proteinuria

\* Contamination with NaCl

In Case C. K., the marked proteinuria was due to glomerulonephritis, was associated with edema and was probably unrelated to the co-existing lymphogranuloma inguinale. The decrease in serum albumin in these cases would, of course, result in a decrease in protein acid-equivalents and hence of itself tend to make  $B - A$  more positive.

*Serum electrolyte partitions in multiple myeloma and in a group of miscellaneous diseases*

The results of serum electrolyte partitions in 6 cases of multiple myeloma are summarized in Table III.<sup>5</sup> In Cases J. B. and A. T., there was definite hyperproteinemia, due chiefly or solely to an increase in euglobulin. The apparent excess of total determined acids over total base in these cases varied from 3.4 to as much as 8.2 m.eq. per liter. In the remaining cases in this group, the acid-base balance was complicated by co-existing renal insufficiency (indicated by definite nitrogen retention, as noted in Table III) with accumulation of sulfates in the blood. In Cases S. E. and G. D., in which hyperproteinemia was not noted, the amount of sulfate in the blood was determined (Table III). It will be seen that adding the values for sulfate to the sum of total determined

acids in these cases still leaves a positive value for  $B - A$ . In Case P. F., in which  $B - A$  appeared to be positive although the serum protein was 9.9 per cent, the amount of sulfate in the blood associated with the marked nitrogen retention present was not determined. It is not known whether, in this instance, total determined acids plus sulfates exceeded the total base.

Table IV summarizes our results on 11 cases of hyperglobulinemia of diverse and, in part, unknown etiology. The results are, in general, consistent with those obtained in lymphogranuloma inguinale and multiple myeloma, the sum of total determined acids exceeding the total base. In Case E. P., in which the euglobulin fraction was within normal limits, and on one occasion in Case A. S.,  $B - A$  was positive. In Case J. D., the equivalents of total determined acids equalled approximately the total base.

The diseases represented are so varied as to suggest that the association of marked hyperglobulinemia with a negative  $B - A$  is a phenomenon of general significance.

#### DISCUSSION

The consistency of the association of the discrepancy in acid-base equivalence with hyperglobulinemia is noteworthy since it has been the experience of this laboratory, using the analytical methods previously described, that  $B - A$  is negative otherwise only occasionally, either in normal subjects or in disease. As to the cause of

TABLE IV  
*Miscellaneous cases with hyperglobulinemia. Serum electrolyte partitions, serum protein fractions*

Case	Age	Sex	Diagnosis	Cl	HCO <sub>3</sub>	Protein	PO <sub>4</sub>	Na	K	Ca	Total base	Total acid	B-A	Non-protein nitrogen	Total protein	Alb- bumin	Glob- ulin	Euglob- ulin
	years			m.eq. per liter	m.eq. per liter	m.eq. per liter	m.eq. per liter	m.eq. per liter	m.eq. per liter	m.eq. per liter	m.eq. per liter	m.eq. per liter	m.eq. per liter	mgm. per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.
J. P.	48	♂	Lymphosarcoma (autopsy)	99.8	21.1	25.4	2.3	136.2	4.4	4.5	137.1*	148.6	-11.5	25	12.1	2.9	9.2	5.0
J. C.	50	♂	Undiagnosed	101.6	29.4	19.8	1.8	136.2	4.4	4.5	117.1	152.6	-5.5	28	9.5	2.2	7.3	
														26	9.3	3.4	5.9	
N. L.	48	♂	Undiagnosed	97.5	25.3	19.9	2.3	133.8	4.2	5.5	145.6	116.0	-0.4	29	9.0	3.4	5.6	1.8
B. S.	37	♀	Undiagnosed	105.0	25.4	20.4	1.4	138.5	3.3	4.9	148.7	152.2	-3.5	27	8.9	4.2	4.7	1.4
H. F.	23	♀	Undiagnosed	100.8	25.9	19.6	2.5	137.7	4.5	4.8	149.0	148.8	-0.2	25	8.8	3.5	5.3	
J. H.	63	♀	Lymphosarcoma (biopsy)	97.3	32.7	20.3	2.6	139.3	5.1	5.4	152.0	152.9	-0.9	32	8.7	4.6	4.1	
A. S.	34	♀	Tb. lymphadenitis (biopsy)	100.8	25.8	19.4	2.1	134.9	4.0	5.0	115.9	148.1	-2.2	21	8.5	4.0	4.5	
				104.3	23.3	18.2	1.9	139.4	4.7	5.4	151.5	119.7	+1.8	29	7.9	3.9	4.0	0.6
A. B.	41	♂	Cirrhosis of liver (autopsy)	100.0	29.7	17.9	2.0	138.7	4.4	4.6	147.7	149.6	-1.9	27	8.4	2.4	6.0	2.1
P. F.	34	♀	Undiagnosed	101.3	24.0	18.7	2.2	140.4	4.5	5.5	152.5	150.2	+2.3	25	8.0	4.3	3.7	0.5
M. A.	39	♀	Leprosy (biopsy)	107.1	25.0	17.3	1.9	134.8	4.9	4.9	150.6	151.3	-0.7	29	7.5	3.7	3.8	1.1
J. D.	23	♂	Cirrhosis of liver, jaundice	100.8	24.5	15.5	1.9	137.8	4.3	4.7	148.8	148.7	+0.1	26	7.2	2.2	5.0	1.7

\* Total base in this case determined by a modification of Fiske's method.

this discrepancy, we are unable for the present to do more than suggest certain possibilities and indicate which seems to us most probable. Changes in pH of the serum or alteration in the content of organic acid radicles of the blood as possible factors may be excluded at the outset, since our analyses give no indication of such alterations in the blood.

Peters and Man (17) presented evidence for the existence of lipoid-chlorine in serum and were able in this manner to account, in part, for negative values for  $B - A$  which they obtain in normal sera. It is possible that the presence of lipoid-bound chlorine might contribute to the apparent excess of total determined acids over total base observed in our cases of hyperglobulinemia. Of interest in this connection is the fact that euglobulin is thought to contain a large, loosely-bound lipoid component. However, none of our cases presented definite hyperchloremia, nor were we able to discern any correlation between the degree of excess of total determined acids over total base and the level of chloride in the blood.

Obviously, negative analytical errors in the estimation of total base (or of sodium where, as in this study, total base is calculated as the sum of determined cations) result in falsely low values for equivalents of base and consequently may cause an apparent excess of total determined acids. In only 3 of our 10 cases of lymphogranuloma inguinale in which  $B - A$  was negative (Table II) was the blood sodium below the minimum normal value obtained by the analytical method used (137.0 m.eq. per liter). The low values for blood sodium in these 3 instances were confirmed by repeated analyses.

Consideration of the data in Table II reveals that in addition to the above-mentioned cases in which the blood sodium was below normal, values for total base tend to be lower in our cases in which  $B - A$  was negative than in those cases in which  $B - A$  was positive. In fact, a fair negative correlation can be made out between total base levels in the blood and the degree of excess of total determined acids over total base. This raises the question as to whether or not the apparent discrepancy in acid-base equivalence noted by us in association with hyperglobulinemia should be at-

tributed to loss of base resulting from disease. This explanation is contrary to the experience of this laboratory in a large number of cases with moderate, or even marked, loss of base occurring in a variety of diseases. With the apparent exception of the cases with hyperglobulinemia presented in this paper, loss of base has been found to be associated almost invariably with a compensatory loss of chloride and bicarbonate so that  $B - A$  remains positive irrespective of the level of total base in the blood. It should be pointed out, moreover, that whereas  $B - A$  has been found to be negative consistently in cases presenting definite hyperglobulinemia, we rarely find  $B - A$  negative in diseases exhibiting a loss in total base, except where there is a concomitant marked increase in serum globulin.

The discrepancy in acid-base equivalence is so constantly associated with hyperglobulinemia, in our experience, as to suggest some relationship between them, particularly since both phenomena are of themselves distinctly uncommon. As already pointed out, the individual electrolyte components of the blood, other than protein, exhibited no obvious deviation from the normal in such cases, except lowering of the sodium and of albumin in some instances.  $B - A$  was invariably positive in cases of lymphogranuloma inguinale or multiple myeloma in which the serum globulin was not increased.

The precise nature of the relation between the apparent excess of total determined acids over total base and hyperglobulinemia is uncertain but the authors attach significance to the fact that in no case of lymphogranuloma inguinale did the serum calcium exceed the limits of normal variation, despite increases in serum protein up to 11.2 per cent. In the absence of hyperphosphatemia, or of any evidence for a decrease in ionized calcium, this fact is interpreted to mean that the added euglobulin increment bound no calcium.

It is, of course, hazardous to draw from the capacity of a globulin to bind calcium any deductions concerning its capacity to bind total base. It is suggested, however, that the apparent discrepancy in acid-base equivalence noted by us in association with hyperglobulinemia would be explained if the serum globulin in such cases bound



significantly less base at the pH of the blood than does normal serum globulin. This would lead to erroneously high values for base bound to protein when the factor ordinarily used to calculate base bound to globulin is applied to such sera.

It will be appreciated that the application to sera with definitely increased globulin content of a factor derived from titration curves of normal serum globulin (7) involves the assumption that the base binding capacity of the serum globulin in hyperglobulinemia is identical at the pH of the blood with that of normal serum globulin. The validity of this assumption is open to doubt. As is well known, any appreciable increase in the globulin content of the serum involves a qualitative as well as quantitative change in serum globulin, since the euglobulin fraction almost invariably constitutes most or all of the added increment. Thus whereas in normal serum, euglobulin may comprise as little as 15 per cent or less of the globulin fraction, in multiple myeloma and in certain chronic infections 60 per cent or more of the serum globulin may consist of euglobulin. There is, moreover, evidence that in such pathological sera, the euglobulin may differ in properties (and, presumably, in structure) from normal euglobulin.

The authors are of the opinion that the excess of determined acids over total base encountered in sera with definitely increased globulin content is apparent only and is the result of erroneously high values for protein acid-equivalents. The error is introduced by the application to pathological serum globulin (which usually contains a large proportion of abnormal euglobulin), of a factor for calculating base bound to globulin which was derived from normal serum globulin.

The problems arising in connection with the estimation of *B* protein in *hyperproteinemia* appear, in fact, to be analogous in some ways to those which came to the fore when acid-base balances were first attempted in blood with *decreased* protein content. Since the decrease in serum protein affected chiefly or solely the albumin fraction, and since *B* albumin greatly exceeds *B* globulin at the pH of the blood, errors were introduced in estimating *B* protein by the use of a common factor which was derived from sera with

normal albumin: globulin ratios. This source of error was eliminated by determining the albumin and globulin fractions and applying to them separate factors for the estimation of *B* albumin and *B* globulin (7).

The data presented in this paper suggest that a further correction of the factor ordinarily used to calculate *B* globulin is necessary when that factor is applied to sera with markedly increased globulin content; otherwise the values obtained for protein acid-equivalents in such sera are too high and *B* — *A* appears to be negative.

#### CONCLUSIONS

1. The concentration of serum proteins was found to exceed 8.0 per cent in 26 of 35 patients with lymphogranuloma inguinale. The euglobulin and pseudoglobulin I fractions, as determined by Howe's method, were increased.

2. Electrolyte partitions of the sera of 18 patients with lymphogranuloma inguinale revealed an apparent excess of determined acid equivalents over total base in those cases presenting definite hyperglobulinemia. A similar apparent discrepancy in acid-base equivalence was observed in association with hyperglobulinemia due to multiple myeloma and, occasionally, to other causes. The apparent discrepancy in acid-base equivalence was not noted in cases of lymphogranuloma inguinale or of multiple myeloma not presenting definite hyperglobulinemia.

3. The discrepancy in acid-base equivalence is probably the result of erroneously high values for protein acid-equivalents; the error being introduced when the factor ordinarily employed to estimate base bound to globulin is applied to sera with markedly increased globulin content.

4. It is suggested that when the factor now in general use for estimating base bound to globulin is applied to sera with definite hyperglobulinemia, a correction is necessary.

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# PLACENTAL INTERCHANGE. I. ON THE CONCENTRATION OF CERTAIN NITROGENOUS SUBSTANCES IN THE BLOOD, BEFORE AND AFTER PASSING THROUGH THE PLACENTA

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Comparative studies on various constituents of the maternal blood before and after its passage through the gravid uterus as well as similar studies on the arterial and venous blood of the fetus have contributed notably to our knowledge of prenatal metabolism and of placental transmission. Evidence is accumulating daily to indicate that the placenta is a complex selective filter which determines what substances shall cross its threshold. Of 73 substances examined by Kehrler (5) in 1907, 43 were found to pass through the placenta. This list has been subsequently amplified as is shown by the compilations of Grulee and Bonar (4), Dogliotti (3), and others. Much of the earlier work was done on the lower animals. In the human species, because of obvious restrictions, such studies have been more limited, being confined almost exclusively to observations made at the time of delivery.

Reference will be made only to several of the more recent publications of special interest. In these the reader may find a fairly comprehensive bibliography which need not be duplicated here.

The exchange of lipids in the umbilical circulation at birth has been recently described by Boyd and Wilson (2). There seems to be general agreement, as expressed by the citations of Naeslund (8), Nevinny (9), Lévy-Solal et al. (7), and others, that the concentration of proteins in the maternal circulation is greater than that of the newborn child. Moreover, Naeslund found the protein concentration to be inversely proportional to the water content of the blood. Lévy-Solal et al. discovered that fetal blood contains a proportionately greater amount of albumin over globulin than does the maternal blood. On the basis of studies on rabbits and dogs, Bickenbach and Rupp (1) concluded that peptides do not pass through the placenta, and that albumin must first

be broken down by placental ferments to amino acids, before passing through. According to Slemmons (11) and Bickenbach and Rupp there is, therefore, no need of assuming that the placenta synthesizes proteins for the fetus. Plass and Matthew (10) and Naeslund (8) are in accord in finding a greater concentration of amino acid in the fetal than in the maternal circulation. Naeslund found that the umbilical arteries carry a greater concentration of amino acids than does the umbilical vein. Bickenbach's findings on this point were inconstant. The nonprotein nitrogen bodies were detected by Plass and Matthew and Naeslund in higher concentration in the fetal than in the maternal circulations. A very slight excess in favor of the maternal circulation was, however, reported by Slemmons.

The present communication describes a portion of a more extended investigation of the general problem of placental interchange. While acknowledging our indebtedness to earlier workers for their valued contributions to this mosaic, it seemed desirable for our purpose to obtain composite blood analyses of a fairly large group of normal subjects. For purposes of correlation, this should have a considerable advantage over determinations of single substances in different groups of subjects. It is hoped shortly to compare these basic findings on normal subjects with those having various toxemias or other complications of pregnancy.

## METHODS

*Subjects.* This study comprises analyses of blood from 40 parturient women, along with those of their respective newborn children. By selecting a fairly large group of individuals, the single variations should be rendered less conspicuous. In every case, a preliminary history and physical

examination established the essentially normal condition of the patient. The patients were not selected with reference to age, race, or previous dietary habits. Because little food is ordinarily consumed during labor, the values obtained probably approach fasting levels. The blood was studied only in vigorous, normal appearing, newborn children weighing at least 3,000 grams.

*Collection of blood.* Blood samples were withdrawn without stasis from a cubital vein of the mother, potassium citrate being used as an anticoagulant. In the 40 cases, the samples were almost equally divided between those collected just prior to actual parturition, during parturition, and immediately following parturition. In no instance did more than 6 minutes elapse between the time of collecting the maternal and the fetal blood. Postpartum blood samples were not taken in cases in which a profuse hemorrhage followed the delivery of the child. Almost immediately after the birth of the child, the umbilical cord was severed, and the blood which spurted from one of the umbilical arteries was saved for analysis. Blood from the umbilical vein was likewise promptly obtained from the maternal end of the severed cord.

*Analyses.* Hematocrit readings were taken after centrifugalization at 3,000 R.P.M. for 30 minutes. Protein determinations (calculated as  $N \times 6.25$ ) were made by the micro-Kjeldahl method using selenium oxychloride as a catalyst. The globulins were salted out from the plasma with 22 per cent potassium sulphate. The non-protein nitrogen was determined by the Kjeldahl method on the Folin-Wu filtrate. Amino acids were determined by the colorimetric method of Folin. To diminish the incidence of summation of errors, all analyses were run in duplicate from the original samples, and an accuracy of 2 per cent in the check determinations was required.

## RESULTS

The findings of this study are summarized in Table I. The extent of the variations in the values determined for the different substances studied is indicated by the coefficients of variation and by the figures showing the standard deviations. Even when allowance is made for these variations, the arithmetical averages or means can be considered as units and serve to illustrate the selective nature of the placental threshold. The low hematocrit of

TABLE I

*Determinations on the blood of 40 normal parturient women, along with those on the blood of their respective newborn children*

	Maternal vein			Umbilical artery			Umbilical vein		
	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum
Hematocrit	40.3	34.84	29.1	51.2	47.0	43.6	50.4	45.85	43.3
Standard deviation		2.52			1.72			1.61	
Coefficient of variation, per cent		7.23			3.66			3.51	
Amino acid nitrogen, mgm. per cent	8.1	6.87	6.1	8.9	8.19	7.50	8.72	8.05	7.30
Standard deviation		.479			.393			.352	
Coefficient of variation, per cent		6.98			4.80			4.38	
Nonprotein nitrogen, mgm. per cent	31.5	27.25	23.1	30.5	28.35	25.7	30.4	27.39	25.2
Standard deviation		1.88			1.47			1.24	
Coefficient of variation, per cent		6.92			5.18			4.54	
Total protein, grams per cent	7.21	6.55	5.94	6.13	5.59	5.34	6.31	5.89	5.46
Standard deviation		.294			.193			.206	
Coefficient of variation, per cent		4.65			3.46			3.50	
Albumin, grams per cent	4.59	3.73	2.90	4.33	3.46	2.52	4.34	3.47	2.58
Standard deviation		.367			.425			.461	
Coefficient of variation, per cent		9.86			12.29			13.28	
Globulin, grams per cent		2.82			2.13			2.42	
Albumin : globulin ratio		1.32			1.63			1.44	

the mother's blood, so familiarly noted during pregnancy, is obvious. The figures tell only of the concentration and not of the absolute number of red blood cells in the circulation. Keith, Rowntree and Geraghty (6), using vital red, found an increase both in the blood and plasma volumes during pregnancy. This fact must be taken into account in the explanation of the apparent anemia. The relatively high hematocrit of the fetal blood is in accord with the generally known polycythemia of the newborn. The greater volume of erythrocytes in the fetal arterial blood as opposed to that in the fetal venous blood speaks for the withdrawal of fluid from the blood during its passage through the fetus, and the subsequent addition of water by the passage of blood through the placenta. That this interpretation may well apply is suggested by the observation of Naeslund that the venous and arterial blood of the umbilical circulation contains 92.1 per cent and 91.9 per cent of water respectively.

Our findings with reference to the amino acids are in general accord with those of Slemons (11), Plass and Matthew (10), and Naeslund (8). The excess of amino acids in the fetal circulation over that in the maternal circulation would suggest absorption or fixation of these acids by the fetus in a manner similar to that described by Van Slyke. This worker observed that amino acids after being injected into the circulation are taken up by the tissues until they contain about 10 times as much amino acid as the plasma.

The nonprotein nitrogen bodies were found to be almost equally represented in the venous and arterial circulation of the fetus as well as in the venous circulation of the mother. This close relationship is hardly incidental, for as waste products they can be expected to diffuse readily through the fetal tissues as well as through the placenta. The slight, though detectable, excess of nonprotein nitrogen in the fetal circulation over that in the maternal circulation may possibly be explained on the basis of a more active metabolism.

The concentration of the maternal plasma proteins is noted to be definitely higher than that of the fetus, this observation confirming that of Naeslund (8), Nevinsky (9), and Lévy-Solal et al. (7). This certainly speaks against any free

passage of protein through the placental barrier. Our findings show a perceptible excess of plasma proteins in favor of the circulation of the umbilical vein over that of the umbilical arteries, the mean values being 5.89 grams per cent versus 5.59 grams per cent, respectively. Naeslund and Nevinsky, in contradistinction, found more protein in the umbilical arterial circulation. It must be admitted, however, that in our series of 40 cases, 11 showed a slightly higher value in the arterial than in the venous blood. The same relationship, with reference to the concentrations of the total plasma proteins in the circulations of the mother, the umbilical vein, and the umbilical artery, is noted to apply to their respective concentrations of albumin and globulin. The globulin values were determined by subtracting the albumins from the total proteins. Hence the correction or variation factors of the globulins are dependent on those of the albumins and total proteins. The albumin:globulin ratios of the circulation of the umbilical vessels would indicate that the plasma of the fetus is richer in albumin than is that of the mother.

#### SUMMARY

Generalizations concerning the passage of substances through the placenta are hazardous without reference to a consideration of specific substances. The evidence would indicate that the placenta is teleologically sensitive in its selection of substances to be added to or withdrawn from the fetal circulation. The nonprotein nitrogen bodies apparently pass through the placenta in either direction according to the rules of simple diffusion. The small size of the amino acid molecules will permit of their passage to the fetus where they are retained in excess. The large molecules of the albumins and globulins do not seem to pass through the placenta to the fetus, and it is assumed that these are synthesized from the amino acids by the fetal tissues. In the interpretations of variations in the relative amounts of substances found in the circulation on either side of the placenta, and between the venous and arterial circulations of the fetus, one must remember to distinguish between the matter of absolute quantity and of concentration of these substances. The significant phenomena associated with water

metabolism and content of the blood must always be regarded in the explanation of results.

The author wishes to express his thanks to Miss Jane Dye for the statistical analyses of data as expressed by her calculations of the deviations from the mean and of the coefficients of variation.

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nitude, while in the dog the ferrocyanide clearance is much the greater.

Shannon (1935) and Shannon and Smith (1935) have determined the inulin clearance in man, and adduce reasons for believing that the inulin clearance approximates the glomerular filtrate. Shannon finds that the creatinine clearance exceeds the inulin clearance, and suggests that the creatinine clearance is higher due to some tubular secretion of creatinine in addition to its excretion by glomerular filtration. From Shannon's creatinine:inulin clearance ratio in man and our creatinine:ferrocyanide ratio we can calculate a hypothetical inulin:ferrocyanide clearance ratio as

$$\frac{\text{Inulin clearance}}{\text{Ferrocyanide clearance}} = \frac{\text{Inulin clearance}}{\text{Creatinine clearance}} \times \frac{\text{Creatinine clearance}}{\text{Ferrocyanide clearance}} = 0.72 \times 2.34 = 1.68.$$

If inulin clearance represents glomerular filtrate in man, the ferrocyanide clearance averages only 1/1.68, or 60 per cent of the glomerular filtrate. Explanation based on the filtration-reabsorption theory could attribute the lower ferrocyanide clearance either to failure, by 40 per cent, of plasma ferrocyanide filtration to equal plasma water filtration, or to tubular reabsorption of 40 per cent of the completely filtered ferrocyanide. The latter explanation seems the more probable. Evidence that ferrocyanide has no difficulty in diffusing through vascular membranes is provided by the observation that the volume of distribution of the injected ferrocyanide in our experiments approximated 24 per cent of the body weight. A similar value had been found by Laviètes, Bourdillon and Klinghoffer (1936) for sucrose, sulfate and sulphocyanate, and was interpreted by them as representing the interstitial fluid volume. It appears that ferrocyanide diffuses readily into the interstitial fluids, but does not enter the majority of the body cells. If any considerable proportion of the ferrocyanide were bound to the serum proteins, or for other reasons were not truly diffusible, a much smaller apparent volume of distribution might be expected. Also, if ferrocyanide passes vascular membranes readily, it may presumably be filtered with ease through the glomerular membrane in the human. It appears that,

whereas in the dog, ferrocyanide is excreted like inulin by glomerular filtration without tubular reabsorption, in man, it is excreted like urea, with about 40 per cent reabsorption.

We are inclined to interpret our results as representing a species difference between dog and man. It should, however, be pointed out that the levels of serum ferrocyanide in our human experiments are distinctly lower than those in the dog experiments of Van Slyke, Hiller, and Miller (1935), and that in this respect the clearances obtained in their experiments were obtained under conditions differing from ours.

The probability that tubular reabsorption of ferrocyanide occurs in man is strengthened by certain renal irritative reactions described below.

#### *Toxic action of ferrocyanide on the human kidney*

Shortly after these experiments were performed, it was noted that the subjects were showing evidences of renal irritation. The two patients who received the smallest amounts of sodium ferrocyanide, equal to two and three times the routine dosage used by Stieglitz and Knight, had been discharged from the hospital and could not be followed further. However, one subject who received five times the routine amount developed a marked albuminuria, accompanied by numerous granular casts, white cells and epithelial cells, and rare red blood cells. The red cells disappeared almost at once, and the albuminuria and leukocytes gradually disappeared during the subsequent two weeks. No decrease in renal function, as measured by the urea clearance test, could be detected in this patient 5 days after the injection of the ferrocyanide, even though the urinary changes were very marked at that time. The other subjects also showed for several days essentially the same pathological changes in the urine to a somewhat lesser degree, even though they had received larger amounts of ferrocyanide. In no case was any systemic reaction noted during or after the injections.

The occurrence of these nephrotoxic reactions is in sharp contrast to their absence after injection of very much larger quantities of sodium ferrocyanide into rabbits by Gersh and Stieglitz (1934) and into dogs by Van Slyke, Hiller, and Miller (1935). The toxic action on the human kidney



the flow of urine in cc. per minute. The absolute clearance values, uncorrected for surface area, are reported.

# RESULTS AND DISCUSSION

Data on the serum concentrations of ferrocyanide, creatinine and urea and the absolute values of their clearances are shown in Table I. The ratios of the simultaneous clearances are given in Figure 1.

These observations give 2.34 as the average creatinine:ferrocyanide clearance ratio. This is in striking contrast to the results of Van Slyke, Hiller, and Miller (1935) on dogs, in which the ratio was consistently in the neighborhood of unity. The contrast between man and dog is further emphasized by the fact that the ratio of the ferrocyanide to the urea clearance in dogs was found by the above authors to average 1.74; the value for this ratio in our 11 observations in man was 1.20. Thus in man the ferrocyanide and urea clearances appear to be of the same order of mag-

TABLE I  
Simultaneous clearances of creatinine, ferrocyanide and urea

Experiment number	Approximate amount of anhydrous $\text{Na}_2\text{Fe}(\text{CN})_6$ injected	Plasma concentration (mgm. per 100 cc. serum)			Clearances $\frac{UV}{B}$		
		Creatinine	Ferrocyanide	Urea	Creatinine	Ferrocyanide	Urea
1 ♂ 69 years	grams 0.55	19.2 18.6	4.83 3.30		121 103	52.8 58.0	
2 ♂ 39 years	0.80	0.7 8.4	5.32 3.20	6.8 7.1	205 252	103.0 88.0	101.0 98.5
3 ♂ 57 years	1.4	15.5	7.8	21.7	172	62.6	34.3
4 ♂ 16 years	1.9	17.0 14.7	10.5 9.05	8.4	220 204	72.5 55.2	89.5 97.3
5 ♀ 59 years	2.8	22.3 21.1	17.0 13.7	17.0 16.2	82.0 106	37.2 39.0	31.0 30.4
6 ♂ 57 years	2.8 before Period I 2.8 before Period II	20.1 12.2	21.1 23.5	18.4 14.8	95.5 102	68.8 63.0	33.3 46.4
7 ♂ 57 years	6.2	21.3 19.5	43.6 34.9	26.4	54.3 65.0	32.9 30.4	26.6 39.4

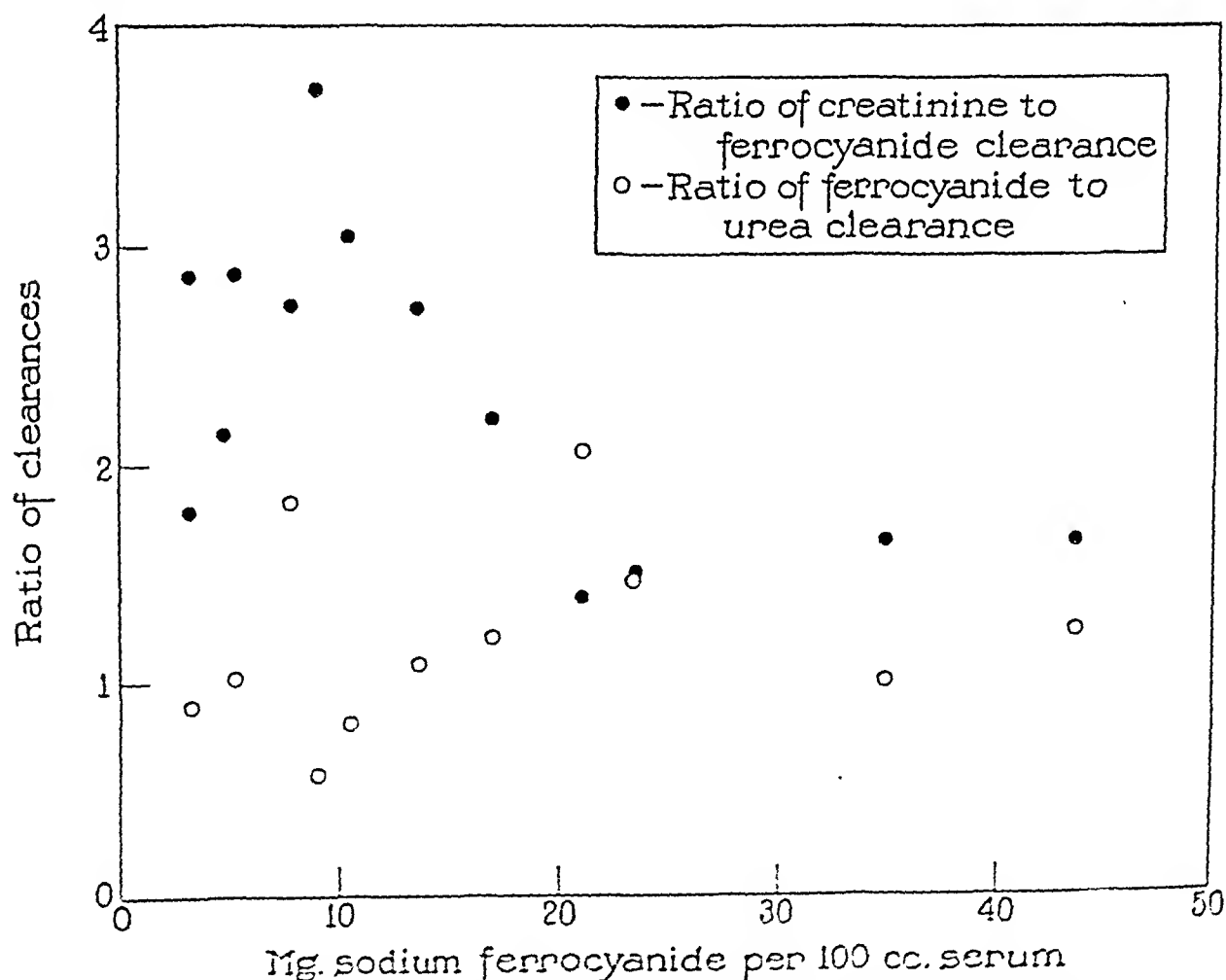


FIG. 1. CLEARANCE RATIOS OF CREATININE: FERROCYNIDE AND FERROCYNIDE: UREA

nitude, while in the dog the ferrocyanide clearance is much the greater.

Shannon (1935) and Shannon and Smith (1935) have determined the inulin clearance in man, and adduce reasons for believing that the inulin clearance approximates the glomerular filtrate. Shannon finds that the creatinine clearance exceeds the inulin clearance, and suggests that the creatinine clearance is higher due to some tubular secretion of creatinine in addition to its excretion by glomerular filtration. From Shannon's creatinine:inulin clearance ratio in man and our creatinine:ferrocyanide ratio we can calculate a hypothetical inulin:ferrocyanide clearance ratio as

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is perhaps due to the difference in its physiological behavior toward ferrocyanide. As shown above, a considerable portion of the ferrocyanide is apparently reabsorbed by the tubular cells. During passage through these cells, the ferrocyanide may well induce toxic effects because of its strong reducing action, and the degenerative changes may become evident later in the urinary changes described above. The urinary findings are consistent with a mild, transient nephrosis caused by chemical toxins. In the dog kidney, where no tubular absorption occurs, no toxic action is produced by the excretion of the ferrocyanide.

In fairness to Stieglitz and Knight (1934), it should be stated that our experiments required larger dosages of sodium ferrocyanide than are advised for their renal function test. However, the very marked nephrotoxic action found after injection of 20 mgm. per kilogram of body weight suggests the *possibility* of harmful reactions with the smaller dosage, especially in patients with renal insufficiency.

#### SUMMARY

The sodium ferrocyanide clearance in man is of the same order of magnitude as the urea clearance, and usually less than half the creatinine clearance. The results, considered with those of Shannon on excretion of creatinine and inulin by man, suggest that ferrocyanide is excreted in the human kidney, like urea, by a process of filtration and reabsorption; and that, on the average, about 40 per cent of the ferrocyanide filtered through the glomeruli is reabsorbed in the tubules.

The excretion of ferrocyanide by man differs sharply from excretion by the dog, which has been shown by Van Slyke, Hiller, and Miller to occur without evidence of reabsorption. Our results

with ferrocyanide, like those of Shannon with creatinine, indicate marked differences in excretory mechanism between dog and man, with regard to these two substances.

Sodium ferrocyanide exerts a definitely toxic action on the human kidney.

The authors are indebted to Drs. Donald D. Van Slyke and John P. Peters for their kind cooperation.

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# PNEUMOCOCCIC INFECTIONS IN FAMILIES<sup>1</sup>

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The development of multiple cases of pneumococcic pneumonia in a family within a short period of time is an infrequent occurrence. When information can be obtained from studies of family outbreaks it is helpful in understanding the epidemiology of pneumococcic infections. In this paper we wish to present an analysis of a series of thirty-three families in which two or more members were ill of pneumococcic infections. In the paper which follows (1) we have recorded bacteriological and immunological studies on all the members of five families of this series.

From the records of patients in whom the type of pneumococcus was determined at The Boston City Hospital during the past fifteen years we have found thirty-three family or contact groups in which two or more individuals were ill within a period of forty-five days. In every instance at least one member of the group had pneumonia. The clinical diagnosis, based on history and physical examination, was confirmed by roentgenogram, lumbar puncture, thoracentesis, or other laboratory procedures. The essential clinical and laboratory data of each case are summarized in Table I.

All the studies reported were made at The Boston City Hospital with two exceptions. One child (Number 23) was admitted to The Children's Hospital. The second patient (Number 16) was cared for at home by one of the district physicians of The Boston Dispensary, and the sputum of this patient was typed in the bacteriological laboratory of the Massachusetts Department of Public Health. We are indebted to the physicians of these two institutions for providing us with abstracts of the case histories.

At the outset certain difficulties were encountered. Only rarely was any attention paid in the

routine history-taking to inquiry into possible contacts with other cases of pneumonia. Moreover, if mention were made of other cases in the family it was frequent that only one member of the family was admitted to the hospital and the others were cared for at home by the family physician whose facilities for bacteriological study obviously were not comparable to those of a well equipped hospital. Occasionally, cases of pneumonia have developed on the wards in patients admitted for conditions other than respiratory infections. For the past several years, for example, an average of two internes of The Boston City Hospital have been ill of pneumonia each year. In many of these instances contact infection has been suspected but since no careful investigation was made at the time, we have excluded all material other than that adequately studied bacteriologically. We present the circumstances under which the material for this study was obtained in order to emphasize the fact that our data do not warrant any conclusions regarding the incidence of multiple cases of pneumococcic infections in families or in contact groups.

In addition to the cases occurring among members of family households we have included two instances of ward contact infection which came under our observation. In the first instance (Number 19) a sister and a brother were ill of lobar pneumonia and bacteremia due to a Type V pneumococcus. A third patient, who was ambulant in the room with the brother, developed a pneumococcus Type V lobar pneumonia, with bacteremia, about twenty-six hours after the initial exposure to the brother. The pneumococcic infection was fatal in all three patients. The ambulant patient had been admitted to the hospital for insulin regulation of diabetes mellitus but otherwise was well. Even though strict vigilance was employed, it was proven that he pillaged beyond the limits of his calculated diet and ac-

<sup>1</sup> This investigation was aided, in part, by a grant given in honor of Francis Weld Peabody by the Ella Sachs Plotz Foundation.

quired his greatest spoils from the trays of other patients too ill to partake completely of their diets. With the apparent occurrence of contact infection observed on this medical ward an investigation was begun for other possible carriers of pneumococcus Type V and forthwith throat cultures were made on all the patients, nurses, and orderlies who had been in contact with either of the two male patients ill of Type V pneumonia. Cultures of the throat of seven patients in the same room, four nurses, and two orderlies failed to yield pneumococcus Type V.

The second instance outside a family group was that of an interne and a patient (Number 21) both of whom, within a period of six days, were ill of a Type VII pneumococcus pneumonia. The patient was admitted on the second day of a lobar pneumonia and a pneumococcus Type VII was isolated from her sputum. The physician, who incidentally had just begun his service, examined the patient, obtained the sputum for typing, and administered specific serum therapy. On the third day after the initial exposure he became ill of an upper respiratory tract infection, and three days

TABLE I  
*Pneumococcic infections in contact and family groups †*

Group number	Relationship	Age	U.R.I.	Pneumococcic infection					Interval between pneumococcic infections	Bacteriology			
				Onset	Admission	Discharge	Diagnosis	Result		Sputum	Blood	Throat swab	Other source
		<i>years</i>							<i>days</i>				
1	Mother	34	0	Jan. 16	Jan. 21	Feb. 3	Lobar	R		I	0		
	Son	2	0	Jan. 16	Jan. 21	Jan. 28	U.R.I.	R	0			I	
2	Brother	20	Jan. 25	Feb. 20	Feb. 21	Mar. 29	Lobar	R		I	0		
	Brother	11	Feb. 1	Feb. 3	Feb. 12	Mar. 1	Lobar	R	17	I	0		
3	Sister	10	0	Nov. 25	Nov. 27	Dec. 14	Lobar	R		I	0		
	Sister	15	0	Nov. 26	Nov. 27	Dec. 14	Lobar	R	1	I	0		
4	Father	68	0	Mar. 10	Mar. 20	Mar. 23	Lobar	D		I	I		
	Son	38	Mar. 27	Mar. 29	Mar. 30	Apr. 24	Lobar	R	10	I	I		
5	Son	13	0	Mar. 4	Mar. 11	Apr. 2	Lobar	R		I	0		
	Mother	45	Mar. 5	Mar. 9	Mar. 11	Mar. 20	Lobar	R	5	I	0		
6	Mother	35	Dec. 23	Dec. 30	Jan. 1	Jan. 25	Lobar	R		I	0		
	Son	14	0	Feb. 1	Feb. 4	Mar. 1	Lobar	R	6(731)	I	0		
7	Father	50	Oct. 1	Oct. 2	Oct. 3	Nov. 3	Lobar	R		I	I		P.F. = I
	Son	5½	?	Nov. 7	Nov. 28	Jan. 1	Emp.	R	4(736)				
8	Husband	62	0	Mar. 6	Mar. 11	Mar. 18	Lobar	D		I			
	Wife	44	0	Mar. 13	Mar. 17	Mar. 25	Lobar	D	7	I	I		
9	Son	16	?	Apr. 13	Apr. 17	May 2	Lobar	R		I	0		
	Mother	41	Feb. 28	Apr. 26	May 1	May 21	Lobar	R	13	I	0		
10	Son	17	Jan. 30	Jan. 31	Feb. 2	Feb. 15	Lobar	R		I	0		
	Father	38	0	Mar. 2	Mar. 4	Mar. 20	Lobar	R	14(730)	I	0		
11	Sister	23	Dec. 31	Jan. 6	Jan. 13	Feb. 24	Lobar	R		I	0		P.F. = I
	Sister	21	?	Jan. 6	Jan. 9	Mar. 18	Lobar-Emp.	R	0	I	I		
12	Son	19	0	Apr. 5	Apr. 8	May 4	Lobar	R		I, VI	0		
	Father	42	Apr. 11	Apr. 12	Apr. 16	May 20	Lobar	R	7	I	0		
13	Sister	6	Jan. 4	Jan. 8	Jan. 11	Apr. 21	Emp.	R					P.F. = I
	Sister	4	Jan. 4	Jan. 9	Jan. 15	Apr. 21	Emp.	R	1				P.F. = I
14	Daughter	5	Dec. 8	Dec. 10	Dec. 24	Mar. 4	Emp.	R					P.F. = I
	Mother	31	Dec. 10	Dec. 20	Feb. 2	Feb. 21	Emp.	R	10				P.F. = I
15	Wife	48	Oct. 12	Oct. 19	Oct. 20	Nov. 26	Lobar	R		II	II	II	S.F. = II
	Husband	56	?	Oct. 24	Oct. 25	Oct. 27	Menin.	D	5				
16	Brother	18	0	Feb. 1	Feb. 3	Feb. 23	Lobar	R		II	0		
	Brother *	13	Feb. 5	Feb. 11			Lobar	R	10	II			
17	Son	15	Dec. 17	Dec. 25	Dec. 28	Jan. 10	Lobar	R		II	0	II	
	Daughter	13	Dec. 23	Dec. 25	Dec. 29	Jan. 10	Lobar	R	0				
	Father	48	Dec. 23	Dec. 26	Dec. 29	Jan. 21	Lobar	R	1	II	II	II	
	Daughter	11	Dec. 19	Dec. 28	Dec. 29	Jan. 14	Lobar	R	3				
18	Sister	24	0	Feb. 2	Feb. 8	Feb. 10	Lobar	D		V	V		
	Brother	29	Feb. 4	Feb. 8	Feb. 11	Feb. 12	Broncho	D		V			
19	Sister	24	?	?	Jan. 12	Jan. 17	Lobar	D		V	0		S.F. = V
	Brother	23	Jan. 12	Jan. 15	Jan. 18	Jan. 25	Menin.	D	3	V	V		
	Ward contact	21	0	Jan. 22	Jan. 22	Jan. 27	Lobar	D	1	V	V		

TABLE I (continued)

Group number	Relationship	Age	U.R.I.	Pneumococcus infection					Interval between pneumococcus infections	Bacteriology			
				Onset	Admission	Discharge	Diagnosis	Result		Sputum	Blood	Throat swab	Other source
		years							days				
20	Son	18	Feb. 18	Feb. 23	Feb. 21	Mar. 3	Lobar	R		V	0		
	Father	36	Feb. 20	Feb. 23	Feb. 26	Mar. 6	Lobar	R	2	V	0		
	Daughter	6	0	Mar. 28	Mar. 28	Apr. 9	? Broncho	R	22 (†28)		0	V	
	Daughter	5	Mar. 18	Mar. 24	Mar. 25	Apr. 6	Lobar	R			0	XXII	
	Son	3	0	Mar. 28	Mar. 28	Apr. 8	Broncho	R	4		0	XXII	
	Daughter	2	0	Mar. 28	Mar. 28	Apr. 8	? Broncho	R	4		0	XXII	
	Daughter	9	0	Mar. 17	Mar. 17	Mar. 29	O.M.A. Tonsillitis	R			0	S.H. XXII	Ear = XXII
	Son	1½ mos.	0	Apr. 1	Apr. 2	Apr. 9	U.R.I.	R				0	
21	Patient	29	Nov. 10	Nov. 22	Nov. 26	Dec. 8	Lobar	P		VII	0		
	Physician	26	Nov. 29	Dec. 2	Dec. 3	Dec. 14	Broncho	R	6	VII, H.I.	0		
22	Husband	45	0	Mar. 9	Mar. 12	Mar. 22	Lobar	R		XII	0		
	Wife	32	?	Apr. 21	Apr. 23	May 15	Lobar	R	30 (†43)	XII			
23	Son**	24	Nov. 5	Nov. 12	Nov. 18	Nov. 21	Lobar	P			0	XIV	
	Father	44	Nov. 14	Nov. 18	Nov. 18	Nov. 21	Lobar	D	6	XIV	XIV		
24	Father	41	0	Dec. 27	Dec. 30	Jan. 23	Lobar	R		II, Gr. IV	0		
	Son	12	0	Jan. 2	Jan. 6	Jan. 22	Lobar	R	5	Gr. IV	0		
25	Father	29	0	Jan. 17	Jan. 21	Feb. 17	Broncho	R		III	0		
	Mother	28	0	Jan. 19	Jan. 21	Feb. 17	Broncho	R	2	Gr. IV, H.I.	0		
	Daughter	19 mos.	0	Jan. 19	Jan. 21	Jan. 29	U.R.I.	R	2	Gr. IV, H.I.			
26	Father	33	0	Jan. 8	Jan. 9	Jan. 31	Lobar	R		I	0		
	Son	12	0	Feb. 11	Feb. 14	Feb. 26	Lobar	R	11 (†34)	II			
27	Wife	72	?	?	Apr. 25	June 10	Broncho	R		III			
	Husband	78	0	Apr. 14	Apr. 21	Apr. 28	Lobar	D	?	Gr. IV			
28	Brother	32	0	Feb. 13	Feb. 20	Apr. 8	Lobar	R		I			
	Brother	36	0	Feb. 21	Feb. 24	Feb. 25	Lobar	D	8	VIII	VIII		
29	Grandson***	6	Dec. 20	Dec. 29	Jan. 5	Jan. 21	Lobar	R					
	Grandmother	60	Dec. 28	Dec. 31	Jan. 11	Jan. 14	Lobar	R	2	VIII	0		
	Grandfather	58	?	Jan. 6	Jan. 11	Jan. 14	Lobar	D	7	XIV	XIV		
30	Husband	38	?	Apr. 9	Apr. 16	Apr. 16	Broncho	D		Gr. IV			
	Wife	36	Apr. 9	Apr. 15	Apr. 16	May 13	Broncho	R	6	Gr. IV			
31	Mother	49	0	Apr. 13	Apr. 19	May 3	Broncho	R		Gr. IV			
	Daughter	18	0	Apr. 13	Apr. 19	May 4	Broncho	R	0	Gr. IV			
32	Wife	29	0	Jan. 15	Jan. 17	Jan. 27	Broncho	R		Gr. IV, H.I.			
	Husband	29	0	Jan. 16	Jan. 17	Jan. 30	Broncho	R	1	Gr. IV, H.I.	0		
33	Son	18	0	Feb. 21	Feb. 24	Mar. 16	Lobar	R		Gr. IV			
	Father	44	Mar. 19	Mar. 23	Mar. 26	Apr. 5	Lobar	R	7 (†30)	Gr. IV	0		

## † Abbreviations:

U.R.I., Upper respiratory infection.

Emp., Empyema.

O.M.A., Acute otitis media.

Menin., Meningitis.

Pn., Pneumococcus (type in Roman numeral).

Gr. IV, Pneumococcus Group IV.

\* Treated at home by Boston Dispensary District Physician.

\*\* Treated at Children's Hospital, Boston.

\*\*\* Treated by family physician.

H.I., Hemophilus influenzae.

S.H., Streptococcus hemolyticus.

S.F., Spinal fluid.

P.F., Pleural fluid.

R, Recovered.

D, Died.

thereafter, or six days after his first contact, he had physical signs and x-ray evidence of bronchopneumonia. A pneumococcus Type VII was obtained from his sputum. The wards to which this physician was assigned had at that time no other case of pneumonia due to a Type VII pneumococcus.

The relationship between the original and subsequent contact cases is shown in Table I. Group IV pneumococci were responsible for the infec-

tion in each of the eight patients of four families. These cases occurred prior to the subdivision of Group IV pneumococci by Cooper et al. (2, 3) and are included in our series only for the sake of completeness. Obviously, no analysis can be made of Group IV pneumococcus cases since the individual patients may or may not have been infected with the same type pneumococcus.

The remaining twenty-nine cases are readily divided into, 1—those in which the infections were

caused by the same type pneumococcus, and 2—those in which the infecting pneumococci were of heterologous types. The distribution of the cases according to the type pneumococcus is shown in Table II. Twenty-three groups, comprising

TABLE II  
*Type distribution of family and contact cases of pneumococcic infections*

Type	Number of groups	Number of patients
I.....	14	28
II.....	3	8
V*.....	3	12
VII.....	1	2
XII.....	1	2
XIV.....	1	2
Homologous.....	23	54
Heterologous.....	6	14
Group IV.....	4	8
Total.....	33	76

\* One family includes a double epidemic involving 3 patients with Pn. V and 4 patients with Pn. XXII (1).

fifty-four individual patients, were ill of pneumococcic infections due to organisms of homologous types, comprising seventy-nine per cent of the cases. In one family (Number 20), included among those of homologous type, there was a double epidemic, the organisms being pneumococci Types V and XXII. This family was studied in detail, bacteriologically and immunologically, and is considered fully in the paper that follows (1).

While in a large majority of the families pneumococci Types I and II accounted for the infections, it is significant that in seven instances pneumococci formerly classified as Group IV were specifically identified in the several members ill of pneumococcic disease. In three families pneumococcus Type V was isolated, and pneumococci Types VII, XII, XIV, and XXII were responsible for the infection each in one family group.

In the individual groups of cases infected with homologous pneumococcus types, the data indicate that when due allowance is made for differences in age, the disease had common features suggesting a similar virulence of infection beyond the presence of the same pneumococcus type. Thus the results of the blood cultures usually corresponded among the relatives, or both members of the same family developed empyema, or the ter-

mination was the same in each patient of the individual group.

The pneumococcic lesions were not always in the lungs. In each family group, however, at least one member had a lobar pneumonia but within the family group the pneumonia was not always of the lobar type, as seen in Numbers 18 and 20 where pneumococcus Type V caused a lobar pneumonia in one patient and a bronchopneumonia in the relative. Numbers 20 and 21 further illustrate that pneumococci Types XXII and VII respectively can produce a lobar pneumonia in one member of a family and a bronchopneumonia in other patients. Extra-pulmonary lesions, namely meningitis (Number 15) and acute otitis media (Number 20), can also be attributed to the pneumococcus of the same type that caused pneumonia within the family group. Upper respiratory infections in contacts who did not develop pneumonia, but from whom a pneumococcus of the same type was isolated from their nasopharynx as from the relative with pneumonia, are presented in Numbers 1 and 20.

In the six groups of patients infected with pneumococci of heterologous types, only one family (Number 26) exhibited both a distinct type difference and satisfactory evidence of contact. In this family, the father had a pneumococcus Type I lobar pneumonia, and the son later developed a pneumococcus Type II lobar pneumonia. Two families are included in which the exposures may have been of less intimate character than in the other instances presented. Number 28 consisted of two brothers, both chronic alcoholic addicts, who developed lobar pneumonia, due to pneumococci Types I and VIII respectively, within an interval of eight days. While the brothers lived in the same house, the extent of their contact with each other was not certain. In Number 29, the grandson was attended three or four hours each day by his grandmother who subsequently developed a Type VIII pneumococcus lobar pneumonia. The grandfather had had no exposure to the child for several weeks and his only contact with his wife, after the onset of her illness, occurred following her admission to the hospital. Seven days after the development of pneumonia in the grandmother, the grandfather contracted a pneumococcus Type XIV pneumonia,

with bacteremia. Of the three remaining groups of cases, two are concerned with Type III and Group IV pneumococci, the etiological significance of which may frequently be in doubt (4, 5) and was not definitely proved in these cases; and in the third family both members had Group IV pneumococci and one had Type II in addition.

In six instances (Numbers 6, 7, 10, 22, 26, and 33) there was difficulty in deciding what should be considered the correct interval of time between the original and the subsequent contact case, inasmuch as there was the question of double exposure. As shown, a patient was admitted to the hospital with a pneumococcic infection, was discharged after a necessary period of convalescence and, following his return home, a second case developed. Because the exact time of the acquisition of the infecting pneumococcus is indeterminate it is impossible to do more than indicate the double exposure. It has been shown (6-10) that either a patient ill of pneumonia or a contact may be a carrier of the causative pneumococcus for as long as six to twelve months. Regardless of which time is taken as the correct period, it is seen that the longest interval between contact cases was forty-three days (Number 22). In the greatest number of groups the onset of the pneumococcic infections was separated by an interval of fourteen days or less, and in three families the onset of the pneumonia was on the same day, strongly suggesting a common source of infection.

#### DISCUSSION

The conception of the dissemination of pneumococci producing disease has undergone considerable change in the last quarter of a century. Before the subdivision of pneumococci into types many investigators assumed that pneumococcic infections were of autogenous nature, based on the finding of pneumococci in the mouths of normal, healthy individuals. In order for disease to develop it was supposed that the resistance of the individual was lowered, or that the virulence of the pneumococcus was increased, or that some combination of the two occurred. Serious doubt was cast on this concept by Dochez and Avery (11, 12) who demonstrated that such an occurrence could account for only a small proportion

of the total number of cases of pneumonia since eighty per cent were due to pneumococci Types I, II, and III, whereas it was rare to isolate pneumococci Types I and II from the sputum of normal individuals. Moreover, they noted when a normal individual harbored Types I, II, or III pneumococci in his sputum, the individual had been in contact with a case of pneumonia and the type corresponded to that with which the patient with pneumonia was infected. Their general conclusion was, therefore, that the spread of pneumonia was the result of direct or indirect contact with a previous case.

Further investigation into the epidemiology of pneumococcic pneumonia has been directed along three main lines, 1—a study of pneumococcus carriers among those exposed to cases of pneumonia and in control series of healthy individuals in the population at large (6-25), 2—investigation of the family groups in which a case or multiple cases of pneumonia have developed (7, 19, 26, 27, 28, 29), and 3—observations on the bacteriology of epidemics of pneumonia (30-35). These studies are obviously of an overlapping nature, and many papers have included all phases. All the reports have emphasized the marked increase in the carrier rate in the group of individuals exposed to cases of pneumonia, and especially is this true in the cases of pneumococci Types I and II infections. Some of the investigators have also succeeded in demonstrating, in family contacts of cases of lobar pneumonia, instances of infections other than lobar pneumonia but due to the same type of pneumococcus.

The present paper adds thirty-three instances of multiple family or contact cases of pneumococcic infections in which the evidence strongly indicates that the subsequent infections were the result of exposure to a previous case or to a common source. It seems highly significant that twenty-three groups, or seventy-nine per cent of the cases, exclusive of those due to pneumococci Group IV, were infected with pneumococci of homologous types, and that over half of these cases were caused by a pneumococcus Type I or Type II. The further classification of pneumococci formerly included in Group IV (2, 3) has made possible the correlation of infections due to these types, which might previously have been



considered to be normal mouth organisms. More adequate bacteriological and immunological studies of cases infected with various pneumococcic types now permit of a more definite evaluation of the disease-producing agent in these cases (4, 5). There are presented here instances of multiple cases of pneumonia and other infections with types other than I and II.

#### SUMMARY

Thirty-three groups of multiple cases of pneumococcic infections are reported in which the evidence indicates that they were the result of contact with an antecedent case. With the exception of two instances, the infections occurred in families. Twenty-three groups included fifty-four individuals ill of pneumococcic infections of homologous types. The majority of contact infections occurred in less than fourteen days after exposure and were of similar severity. While the causative organisms were most frequently the Type I and II pneumococci, a significant proportion of the instances of multiple cases of pneumonia were due to type-specific pneumococci formerly classified in Group IV. Instances of empyema, primary meningitis, acute otitis media, simple upper respiratory tract infections, and bronchopneumonia were encountered in which the causative pneumococcus was of the same type as that which gave rise to uncomplicated lobar pneumonia in other members of the family.

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# BACTERIOLOGICAL AND IMMUNOLOGICAL STUDIES IN FAMILIES WITH PNEUMOCOCCIC INFECTIONS: THE DEVELOPMENT OF TYPE-SPECIFIC ANTIBODIES IN HEALTHY CONTACT CARRIERS<sup>1,2</sup>

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The occurrence of multiple cases of pneumonia due to the same type of pneumococcus within a family or among persons in contact with cases and the presence of milder respiratory tract infections associated with the same organism among the contacts of pneumonia cases were recorded in the previous paper (1). These observations, coupled with the high incidence of healthy carriers of the same type of pneumococcus among family contacts of persons ill with pneumonia, suggested an opportunity for studying the immune response to the milder infections with these disease-producing pneumococci as well as the response to their presence in the pharynx when they do not give rise to clinical symptoms. In this paper are presented the results of more detailed bacteriological and serological studies in the patients and other members of the household of 5 of the 33 families previously reported (1) in which multiple cases of pneumococcic infection occurred.

## MATERIAL AND METHODS

*Bacteriological studies* were directed mainly to the isolation and serological identification of pneumococci. Sputum, blood, pleural fluid, discharges from the ear, and pharyngeal swabs were obtained from cases and cultured (2). Pharyngeal swabs from healthy contacts or from patients who raised no sputum were placed into rabbits' blood broth, incubated for a few hours, and the resulting growth inoculated into mice. These blood broth cultures, the peritoneal exudate of the mouse at various intervals, and the mouse heart's blood were streaked on the surface of blood agar plates, and numerous suspicious colonies picked, isolated in pure culture, and identified

by morphological and cultural characteristics, by bile solubility, and by agglutination with antipneumococcic sera for Types I-XXXII (Cooper).

*Immunological studies* consist of tests for type-specific agglutinins and mouse protective antibodies in the blood serum. The protection tests were carried out only with the types for which cultures of maximal virulence were available. Attempts to raise the virulence of a Type XXII culture by repeated mouse passage were not successful, the lethal dose remaining less than 0.000,001 cc. Tests for agglutinins were carried out with formalized suspensions of pneumococci. When floccular agglutination could not be demonstrated, stained smears of fresh 12-hour blood broth cultures mixed with the test serums were examined for the presence of microscopic agglutination.

While it was hoped to obtain early and frequent cultures and bloods from all contacts, this was possible only in some of the members of one of the families studied. Single cultures and serum samples were obtained in the others.

## CLINICAL, BACTERIOLOGICAL, AND SEROLOGICAL OBSERVATIONS

*Family McD.* (Table I) had two members admitted to the hospital, both of whom had lobar pneumonia followed by empyema due to a pneumococcus Type I. The first manifestation of infection in family McD., however, was in the mother who contracted a mild irritative cough in November. She was asymptomatic aside from the cough which persisted almost unchanged for 2½ months, that is until the middle of January. The eldest daughter, Ma., age 8 years, developed an upper respiratory tract infection on December 31, with fever, cough, and malaise. Ma. was not ill enough to go to bed but continued her usual routine and was well after a three day illness. On January 4 both C. and T. showed evidence of a cold, and on January 8 and 9, respectively, they had identical symptoms indicative of a more severe infection, namely vomiting, fever, prostration, and epigastric pain. They were subsequently admitted to The Boston City Hospital with lobar pneumonia and later had empyema. Type I pneumococci were isolated from the pleural fluid of both C. and T. Throughout the period of respiratory infections in the family, the father remained quite well. Throat cultures were made on February 3 on the father.

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<sup>2</sup> This work was carried out with the technical assistance of Mrs. Mildred W. Barnes.

TABLE I \*  
Family McD. (Number 13)

Patient	Age	Relation-ship	Clinical	Bacteriology			Immunology					
				Date	Throat swab	Other	Agglutinins				Protection	
							Date	I	XI	XXIV	I	V
Th.	46	Father	Well	Feb. 3	XI, XXIV		Feb. 3	0	+	+	0	0
Mo.	35	Mother	Nov. 25, cough for 10 weeks	Feb. 3	I		Feb. 3	0	0	0	500	0
Ma.	8	Daughter	Dec. 31, fever, cough, 3 days	Feb. 3	I		Feb. 3	0	0	0	5000	0
C.	6	Daughter	Jan. 8, lobar pneumonia, empyema	Jan. 15		P.F.=I						
T.	4	Daughter	Jan. 9, lobar pneumonia, empyema	Jan. 16		P.F.=I						

\* Explanation of Tables I to IV

Number after family designation corresponds to group number in Table I of previous paper (1). Clinical: dates refer to onset of symptoms. Bacteriology: Roman numerals represent pneumococcus types; P.F. = pleural fluid; S.V. = *Streptococcus viridans*, S.H. = *Streptococcus hemolyticus*. Immunology: Date represents withdrawal of blood for serology; agglutinins: + = microscopic agglutination present but negative macroscopically; 1:2, 1:4, etc. = greatest dilution showing floccular agglutination; protection = mouse protective antibodies in lethal doses per cc.; - = not done.

mother, and daughter Ma., at which time venous blood was drawn for immunological studies. The data obtained are shown in Table I. Both the mother and daughter Ma., who had had mild respiratory infections, were found to be carriers of a Type I pneumococcus while the father who had had no clinical evidence of infection did not harbor a Type I pneumococcus in his throat but carried pneumococci Types XI and XXIV. The father's serum showed microscopic agglutination against pneumococci Types XI and XXIV but not against Type I. The blood of the mother and daughter Ma. had no demonstrable agglutinins, either microscopically or macroscopically, against pneumococci Types I, XI and XXIV. The father's serum showed no protective antibody against pneumococcus Type I while protection against this type was demonstrated in the blood serum

of both the mother and the daughter, Ma. Control protection tests against pneumococcus Type V were negative in these 3 individuals.

*Summary.* Family McD. consisted of five members, two of whom had a pneumococcus Type I empyema, two of whom had upper respiratory infections and were carriers of pneumococcus Type I, and one of whom remained well and did not carry a Type I pneumococcus. Protection against pneumococcus Type I was demonstrated in the blood serums of the carriers of Type I but not in the blood of the father who was not a Type I carrier.

TABLE II  
Family L. (Number 16)

Patient	Age	Relation-ship	Clinical	Bacteriology				Immunology						
				Date	Spu-tum	Throat swab	Blood	Date	Agglutinins					Protec-tion
									II	III	X	XIX	XXI	
S. L.	18	Son	Feb. 1, lobar pneumonia, serum therapy	Feb. 3	II		0	Feb. 3 Feb. 4	0 1:32					
N. L.	13	Son	Feb. 11, lobar pneumonia	Feb. 12	II			Mar. 1	1:16	0	0	0	0	50,000
F. L.	50	Father	Well	Mar. 1		X, XXI		Mar. 1	0	0	0	0	0	0
M. L.	45	Mother	Well	Mar. 1		III, XIX		Mar. 1	1:2	0	0	0	0	5,000

*Family L.* Table II gives the data regarding this family in which two brothers were ill of lobar pneumonia. S., without preceding infection of the upper respiratory tract, suddenly on February 1 had the classical symptoms of a lobar pneumonia and was admitted on February 3 to The Boston City Hospital with physical signs and x-ray evidence of involvement of the left lower lobe. Because of the finding of a pneumococcus Type II in his sputum, he was treated with specific antipneumococcus serum and made a rapid, uneventful recovery. On February 5, N., the brother, who slept in the same bed with S., developed malaise, coryza, and dizziness. N. became worse on February 11, ten days after the onset of the pneumonia in S., with chest pain, temperature of 103° F., "rusty" sputum, and a diagnosis of pneumonia of the right upper lobe was made by one of the district physicians of the Boston Dispensary. From N.'s sputum a pneumococcus Type II was isolated (1). N. made an uncomplicated recovery. Throat cultures were made on the father and mother on March 1, which failed to yield pneumococcus Type II, but did show pneumococci of other types. Also, on March 1 blood was obtained from N., from the father, and from the mother. It is seen in Table II that N. had agglutinins in a titer of 1:16 against pneumococcus Type II and possessed mouse protective antibodies in high titer against this organism. N. had no agglutinins for the types of pneumococci isolated from his mother and father. The father had no demonstrable agglutinins against his own organisms or against the pneumococci from the mother and sons. Likewise the father's blood

serum contained no mouse protective antibodies for pneumococcus Type II. In the mother's blood serum agglutinins in low titer and mouse protective antibodies were present against pneumococcus Type II but agglutinins could not be demonstrated for the pneumococci isolated from her and her husband.

*Summary.* Two brothers of Family L. had Type II pneumococcus pneumonia. Neither the father nor the mother was shown to be a carrier of Type II pneumococcus but each carried pneumococci of different types. The mother possessed agglutinins and protective antibody against pneumococcus Type II but not against her own mouth organisms. The blood serum of the father contained no demonstrable antibodies for any of the pneumococci isolated.

*Family D.* (Table III) presents an instance of the development of lobar pneumonia, due to pneumococcus Type II, in four members of a household within a period of three days. All those ill of pneumonia had had preceding upper respiratory tract infections. All were admitted to The Boston City Hospital and all recovered without postpneumonic complications. Two, the father and the son, were treated with specific antipneumococcus serum. The mother and three other children, as shown in Table III, either remained well or suffered mild colds

TABLE III  
*Family D. (Number 17)*

Patient	Age	Relation- ship	Clinical	Bacteriology				Immunology					
				Date	Spu- tum	Throat swab	Blood	Date	Agglutinins			Protection	
									I	II	XXVII	I	II
J.	15	Son	Dec. 25, lobar pneumonia, serum therapy	Dec. 28 Jan. 7	II II			Dec. 28 Dec. 29 Jan. 6		0 1:32 1:8	0		
M.	13	Daughter	Dec. 25, lobar pneumonia	Dec. 29		II		Jan. 6		1:64			
P.	48	Father	Dec. 26, lobar pneumonia, serum therapy	Dec. 29	II		II	Dec. 29 Dec. 30 Dec. 31 Jan. 6 Jan. 13		0 1:32 1:64 1:16 0	0 0 0 0		
A.	11	Daughter	Dec. 28, lobar pneumonia	Dec. 29		II		Dec. 31 Jan. 8		0 1:32			
R.	?	Mother	Well	Jan. 1		S.V.							
W.	10	Son	Dec. 26, cough, 2 days	Jan. 1		XXVII		Jan. 9	0	0	0	0	0
P., Jr.	9	Son	Dec 31, cough, 1 day	Jan. 1		II		Jan. 9	0	+	0	0	50,000
Ma.	7	Daughter	Well	Jan. 1		II		Jan. 9	0	1:8	0	0	50,000

hospital a few hours after the onset of a bronchopneumonia. Pneumococcus Type XXII was again isolated by throat culture on March 29. W.'s blood serum of March 29 contained no agglutinins or protection against pneumococcus Type V but microscopic agglutinins for pneumococcus Type XXII were present. A throat culture on April 16 showed only pneumococcus Type VI.

Pa. P., age 2 years, became ill on the same day as D. and W. and was admitted to the hospital on March 28. She had a fever of 102° for two days and was thought clinically to have a bronchopneumonia. A pneumococcus Type XXII was obtained from Pa. by throat swab. On March 31 she developed an acute otitis media; the ear drum was incised and a culture of the pus on April 1 yielded a pure growth of pneumococcus Type XXII. Her blood serum of March 29 contained no agglutinins for pneumococci Types V and XXII and no protection for Type V.

H. P., a daughter of 9 years, was a carrier of pneumococcus Type V on March 1. On March 4 she had microscopic agglutinins and protection for pneumococcus Type V, and also her serum on that date had microscopic agglutinins for Type XXII pneumococcus though a Type XXII was first obtained from H.'s throat on March 14. H. became ill on March 17 and was admitted to the hospital on the day of onset with an acute follicular tonsillitis. Throat cultures on March 17 contained a hemolytic streptococcus and a pneumococcus Type XXII. Her blood serum on March 17 showed agglutinins for pneumococci Types V and XXII and protection against Type V. A pneumococcus Type XXII was present in her throat on April 16.

The infant son, R. P., 1½ months old, was admitted to the hospital with a mild infection on April 1 but no pneumococci were obtained from his throat culture.

There were five remaining members of family P. who were not admitted to the hospital. Three throat cultures were made on the mother, Mo. P., but pneumococci were obtained only once, namely a Type V on March 14. The mother's blood had agglutinins and protection for pneumococcus Type V on March 14 but contained no agglutinins against Type XXII. A second blood from Mo. was studied on March 25, at which time the titer of the protective antibodies for pneumococcus Type V was found to have increased but there were no agglutinins for pneumococci Types V and XXII. The mother throughout the entire period of study was free of respiratory infection.

The grandmother, G. O., who lived temporarily in the same house, had a mild sore throat from February 21 to February 28 but was not confined to bed. On February 28 and again on March 14 a *Streptococcus viridans*, but no pneumococci, was isolated by throat culture. Her blood serum of March 14 contained no agglutinins for pneumococci Types V and XXII and no protective antibodies for Type V.

J. P., aged 12 years, remained well. Her throat culture on February 26 yielded a pneumococcus Type V. On March 3 there were agglutinins and protective anti-

bodies for pneumococcus Type V in her blood serum but no agglutinins for Type XXII. The throat culture of March 14 showed pneumococci Type XXII, on March 26 no pneumococci were isolated but on April 16 a pneumococcus Type XXII was again found. The blood serum of March 26 contained no agglutinins for pneumococci Types V and XXII but the protection for Type V had increased over its previous level.

F. P., age 11, who remained entirely free of respiratory infections, was the first member of the family from whom a pneumococcus Type XXII was obtained. On March 4 his throat culture showed pneumococcus Type XXII and his blood serum of that date had Type XXII agglutinins in a titer of 1:64 but contained neither agglutinins nor protective antibodies for pneumococcus Type V. On March 14 both Type V and Type XXII pneumococci were present in his throat cultures and agglutinins were demonstrable in his blood serum of March 14. Between March 4 and March 14, moreover, he had developed a protective titer of 5000 lethal doses per cc. for pneumococcus Type V. The throat culture of April 16 contained only pneumococci of Type XXII, but not Type V or XXII.

Ma. P., 15, had a slight coryza and cough from February 14 to February 28 but was not ill enough to go to bed. On March 1 culture of her throat showed pneumococci Types V and VI. On March 14 a pneumococcus Type V was obtained and on April 16 no pneumococci were grown on culture. Blood serum of March 2 and of March 14 contained no agglutinins for pneumococci Types V, VI and XXII and no protective antibodies for Type V.

After the finding of a pneumococcus Type XXII in certain members of Family P. apparently as the etiological agent of their disease, the original blood serums of B. and Pe. were tested for the presence of Type XXII agglutinins. The serums of B. of February 25 and March 14 were negative but that of March 3 possessed a titer of 1:4. These agglutinins could not be demonstrated in the serum of March 14. Opportunity for further throat cultures on B. was not available. Pe.'s blood serum contained no Type XXII agglutinins on February 26 but on March 4 and March 14 they were demonstrated in low titer. A throat culture of Pe. on April 16 showed a pneumococcus Type XXII.

*Summary.* Family P. presents a complicated double epidemic of infections, pneumococci Types V and XXII. Three members, B., Pe., and D., suffered from pneumonia due to the Type V pneumococcus. Three members, G., W., and Pa., had bronchopneumonia due to pneumococcus Type XXII and one of these had an otitis media due to pneumococcus Type XXII. One, H., had a follicular tonsillitis during which hemolytic streptococci and some Type XXII pneumococci were cultured from the throat. In the five mem-

bers of the family who either remained well or who had mild colds, pneumococci were obtained from the throat at different times from all except the grandmother, G. O. Antibodies were demonstrated against pneumococci Types V and XXII in the individuals who were carriers, with the exception of Ma., though the antibody response was more regular against Type V than against Type XXII. In one individual, F., who remained well, antibodies against pneumococcus Type V developed after a Type V pneumococcus was obtained by throat culture. Less clearly the same thing is seen in the case of the mother, though unfortunately an early blood could not be obtained. There were also two instances in which agglutinins for Type XXII apparently developed under observation during convalescence from Type V pneumonia. The Type XXII was cultured from the throat of one of these cases some time later, and, in the other, no further cultures were made. In H. P., Type XXII agglutinins were demonstrated first, and this pneumococcus was isolated from the throat ten days later.

*Family H.* Detailed studies were made in group Number 19 (1) which consisted of a brother, J. H., a sister, M. H., and a ward contact all of whom had a rapidly fatal Type V pneumococcus pneumonia with bacteremia. The ward contact, H. C., was an ambulant diabetic patient who had acquired a pneumococcus Type V pneumonia and bacteremia as a result of exposure to J. H. who was ill on the same medical ward. Throat cultures made on the seven patients in the same rooms with J. H. and H. C., and also on four nurses and two orderlies assigned to that ward, failed to reveal any carriers of pneumococcus Type V. Agglutinins and mouse protective antibodies against pneumococcus Type V could not be demonstrated in any of the seven patients whose blood serum was studied. Because of the completely negative results in the ward contacts where reasonable precaution was exercised this group is not considered further.

#### DISCUSSION

Previous investigations of outbreaks of pneumonia in families, institutions, or communities (1) have been concerned with the incidence and spread of disease-producing pneumococci among

cases and their contacts. The Types I and II were the only pneumococci concerned in these epidemics except in the institutional outbreak reported by Schroder and Cooper (4) during which Type V pneumococci were recovered from 7 of the 9 cases of pneumonia which were "typed." During this epidemic there was a high incidence of colds and bronchitis but these were not studied bacteriologically. Other investigators have observed the occurrence of simple upper respiratory tract infections among contacts of cases of Type I or II pneumonia and have noted further that some of them became carriers of these pneumococci; but, with the exception of Joppich (5), they failed to ascribe to them any etiological relationship. Immunological studies have been made during these epidemics only in isolated cases of pneumonia, and then only for the purpose of confirming the type when the patients were first seen during convalescence and the causative organisms had not been identified previously (6).

The observations here presented confirm the finding that a high percentage of the contacts within the family of cases of pneumococcus pneumonia harbor the homologous type pneumococcus in their pharynx. This was shown to be true not only for Types I and II but also for Type V and for the less common Type XXII. It may further be deduced from the data in these families that even healthy contacts may develop specific antibodies in response to the presence in their pharynx of the types of pneumococci which produced disease in their relatives.

It is difficult to find, in the literature, examples of a definitely demonstrated development of specific antibody resulting from proved contact with virulent bacteria without intervening infection or the exhibition of antigenic material. Even with diphtheria immunity, the observations of Garrido-Morales and Mandry (7), though seemingly convincing, have failed to meet the objection that the simple performance of the Schick test may be an effective stimulus to antitoxin production in a person with a "basal immunity" (8, 9). On the other hand, many observers have noted the coexistence of virulent organisms and circulating antibody as, for example, Schick noted active carriers of virulent diphtheria bacilli. However, in the case of diphtheria and



fever which are the diseases usually studied, a degree of immunity similar to that found in such individuals is normally to be expected in a considerable percentage of the population. With type-specific pneumococci, on the other hand, antibody demonstrable by the agglutination test as here performed, or by the mouse protection test in the case of most virulent strains, is rarely encountered in normal human subjects.

In this paper there are presented examples of the appearance of antibody for homologous types of pneumococci after their appearance in the pharynx. Furthermore, the presence of type-specific antibodies in moderately high titer for the homologous type of pneumococcus in most of the healthy contact carriers of the disease-producing pneumococci, the absence of antibodies for other pneumococcus types in the same individuals, and the failure to demonstrate similar antibodies in other contacts in the same family who did not become carriers of the same type, all add convincing evidence for the antigenicity of the carrier state in the case of the disease-producing pneumococci.

These findings, when considered in conjunction with the known transient character of the specific antibodies found in patients convalescent from infections with pathogenic pneumococci, would seem to indicate that when agglutinins or protective antibodies for specific pneumococcus types are demonstrated in the serum of an apparently healthy individual, they may have arisen as a result of previous infection or of a carrier state arising out of contact with the same or related type of pneumococcus.

The absence of demonstrable antibodies for most of the types of pneumococci found in the normal pharynx may then be the result of, 1—the long duration of the contact and, hence, the disappearance of the demonstrated antibody, or 2—the low antigenicity of those particular types, or 3—the poor antigenic reaction on the part of the carrier.

How far the antibodies resulting from the carrier state render the individual less susceptible

to pneumonia or to other serious pneumococcal infections can not be answered by the data at hand. Those infections which developed under our observation were due to organisms other than the one for which immunity had been previously demonstrated.

#### SUMMARY AND CONCLUSIONS

Bacteriological studies made on all the members of the household and of some other contacts in five families in which multiple cases of pneumococcus infection were observed revealed a high incidence of carriers of the type of pneumococcus responsible for the infection in the relatives. Serological studies of the carriers and non-carriers showed that homologous type-specific antibodies may develop in a large percentage of healthy contact carriers of disease-producing pneumococci.

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# EXPERIMENTAL INDUCTION OF ERYTHEMA NODOSUM<sup>1</sup>

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The syndrome of erythema nodosum is known to occur in association with a number of diseases (1). The erythematous nodules are strikingly similar both in their gross and microscopic appearance, irrespective of the infection with which they are associated. This similarity of appearance suggests that the lesions may result from a common underlying mechanism. The nature of this mechanism is unknown. However, it has been pointed out that patients with erythema nodosum manifest an unusually intense skin reactivity to products of the bacterial agents responsible for their infection (2, 3). Whether this phenomenon has any significance in the development of the lesions remains to be determined. The present report deals with experimental observations which throw some light on the relationship between intense skin reactions to specific bacterial protein and the development of this syndrome.

Twenty-two patients with erythema nodosum have been under our observation in the past ten years. Two of these individuals had x-ray evidence of pulmonary tuberculosis, reacted strongly to tuberculin, were negative to hemolytic streptococcus nucleoprotein and had normal antistreptolysin titers (33 units on repeated determinations). The other 20 patients reacted strongly to hemolytic streptococcus nucleoprotein, weakly or not at all to tuberculin and had elevated antistreptolysin titers varying from 125 to 2500 units with a median of 500 units. Twelve patients who have been studied in detail are the subject of the present paper.

*Subjects selected.* Eleven girls or young women and one boy<sup>2</sup> were selected for special study. All had high antistreptolysin titers, ranging from 250 to 2,500 units with a median of

500 units. All had recent or subsiding erythema nodosum on both legs, symmetrically distributed.

*Procedure.* All were given hemolytic streptococcus nucleoprotein fraction C 19 K (ref. 2, page 228), intracutaneously on the volar surface of the forearm. Some were given, in addition, nucleoprotein of hemolytic staphylococcus, Pneumococcus I, Pfeiffer bacillus and *B. catarrhalis*. All doses were standardized to contain .001 mgm. nitrogen.

## RESULTS

*Primary reactions.* All subjects developed severe local inflammatory reactions to the nucleoprotein of hemolytic streptococcus, weak or negative reactions to the other bacterial proteins. These reactions began in 12 hours, were intense in 24 hours, reached their height in 48 hours and began to subside in 72 hours. Scaling and induration followed and persisted for a number of days.

*Secondary reactions.* Six of the 12 individuals developed a recrudescence of erythema nodosum following the skin test. The recrudescence began in each instance 36 hours after the skin test; that is, about 24 hours after the beginning of the inflammatory reaction at the site of inoculation. The nodules persisted for 24 to 72 hours and were entirely gone by the time that the inflammatory reaction on the arm had subsided. Nodules on the lower part of the legs appeared first and subsided last. None of the induced recrudescences were as severe as the original attacks.

It was possible to repeat these artificially induced attacks a second time on each subject, by a new injection of fraction C 19 K on the other arm. The second injection of C 19 K did not cause the original site of injection to flare up in a single instance. General skin reactivity to C 19 K was undiminished in these patients after six months, and was still present at the end of one year. However, after a period of two or three weeks it was no longer possible to induce

<sup>1</sup> The work reported in this communication was carried out under the W. K. Kellogg Foundation Fund.

<sup>2</sup> This sex distribution is characteristic of the incidence of erythema nodosum, both in our group and that reported by Edström (1).

a recrudescence of erythema nodosum on the legs.

Two illustrative case histories are presented.

*Mary M., History Number 278407*

The patient, an 8 year old girl of Irish-American parentage, was admitted to the hospital with erythema nodosum of two weeks' duration. Her father had had rheumatic fever, and she had had erythema nodosum at the age of five. Her history was free of frank rheumatic manifestations. On physical examination mild pyrexia, tachycardia and fading erythema nodosum over the lower anterior third of each tibia were present.

*Laboratory findings.* X-ray of chest was negative; x-ray of sinuses showed multiple sinusitis; throat culture showed no hemolytic streptococci; white blood count was 12,000 and blood sedimentation rate 55 mm. in 1 hour; antistreptolysin titer was 500 units on repeated determinations; Mantoux and Dick tests were negative.

The left maxillary sinus was irrigated just before the patient's discharge from the hospital. The throat culture at this time contained no hemolytic streptococci; material from the sinus, however, contained beta hemolytic streptococcus Type XIII in almost pure culture.

*Skin test. Primary reaction.* Three days after admission, when it was seen that no new lesions had appeared on the legs, the patient was given an intracutaneous test with 0.1 cc. of hemolytic streptococcus nucleoprotein fraction C 19 K (containing 0.01 mgm. nitrogen per cc.). The reaction to this test at 24 hours was 8 cm.  $\times$  4.5 cm., erythematous, indurated, hot, extremely painful with a vesicular center. The skin test was repeated seven days later and a similar reaction occurred, measuring 9 cm.  $\times$  7 cm.

*Secondary reaction.* About 36 hours after the first skin test of C 19 K, erythema nodosum developed on both legs. These lesions persisted for 48 hours and then became purple and scaled. As no additional lesions appeared, the skin test was repeated after a few days on the opposite arm. The evolution of the lesions on the legs was observed with great care. The sequence of events following injection was as follows.

- 24 hours: indurated area felt on lower third of left leg, nothing seen.
- 36 hours: one large and two small pink, tender, nodular lesions on medial, lower third of left leg.
- 48 hours: new lesions appeared on middle third of both legs and on lower third of right leg. Old lesions more pronounced.
- 60 hours: new lesions on knees and just above knees (almost symmetrical).
- 72 hours: induration more marked, color fading.
- 84 hours: all lesions faded except those on lower left leg.
- 96 hours: lesions on lower third still palpable. Skin test subsided.
- 5th day: slight induration still present on lower third of legs.
- 6th day: legs returned to previous state.

Skin tests were then done with equivalent quantities of other bacterial proteins. The primary reactions were read as follows:

	24 hours	48 hours
Pfeiffer bacillus .....	$\pm$	—
Hemolytic staphylococcus .....	$\pm$	—
<i>B. catarrhalis</i> .....	$\pm$	—
Pneumococcus Type I .....	$\pm$	—

No secondary reactions followed these injections. One month later the patient was retested with C 19 K and again developed an intense local reaction, and a rise in temperature to 104° F. However, no nodules appeared on the legs after this injection. The sites of previous cutaneous tests did not flare up at any time.

It was not possible in this first patient to determine whether the artificially induced lesions represented new nodules or a recrudescence of old nodules. In the second patient the induced nodules appeared to arise at new sites.

*Evelyn S., History Number 480647*

This patient was also an 8 year old girl who was admitted with erythema nodosum of 1 week's duration on both legs anteriorly and both arms posteriorly, symmetrical in distribution. Like the first patient she had had no frank rheumatic manifestations. Laboratory tests were negative except for leukocytosis of 16,000, blood sedimentation rate of 86 mm. in 1 hour and antistreptolysin titer of 500 units on repeated determination. She gave a positive skin reaction only to the nucleoprotein fraction of C 19 K. The primary lesion measured 11.5 cm.  $\times$  5.5 cm., 24 hours after injection. Thirty-six hours after the skin test she developed a nodule on each leg in an area which appeared not to have been involved previously. These nodules began to fade after 48 hours.

## DISCUSSION

These twelve patients with erythema nodosum developed a severe inflammatory reaction following the intracutaneous injection of hemolytic streptococcus nucleoprotein, at a time when there was a high titer of antistreptolysin in the circulation. The intense local reaction was similar to that seen in other patients who have recently had hemolytic streptococcus infection. However, shortly after the appearance of intense inflammation at the site of the intracutaneous test, six of these children developed a recrudescence of erythema nodosum in the previously affected parts. This response was not elicited unless the local inflammatory reaction was severe. It did not occur in control patients without erythema nodosum. The capacity of the involved extremities to de-

velop erythema nodosum persisted for only a few weeks, at the end of which time an almost identical local reaction had no secondary effect. The evolution of the induced lesions and their relationship in time to the primary skin reaction showed a striking similarity in these six individuals.

Ernberg (3) observed a similar sequence of events in five tuberculous patients with subsiding erythema nodosum. Old tuberculin injected intracutaneously 6 to 14 days after the subsidence of the spontaneous erythema gave rise to intense local reactions which were followed by fresh efflorescences of nodular lesions in the same areas as had been involved previously. His findings were substantiated by Collis (4) who suggested that erythema nodosum is "produced by a soluble break-down product of certain organisms acting on already hypersensitive tissues."

The mechanism underlying the evolution of erythema nodosum is unknown. The circumstances under which the experimental induction of these lesions was possible suggest the following hypothesis. In the course of certain infections, symmetrical areas of the body become conditioned for a few weeks. Intense localized inflammation, presumably the result of specific antigen-antibody reaction, liberates some substance into the circulation. This substance induces non-specific nodular lesions in the conditioned zones at a distance from the localized inflammatory reac-

tion. This hypothesis is consistent with Topley's (5) conception of "the secondary effects of primary cellular reactions."

#### SUMMARY

The intracutaneous injection of the appropriate antigen in a patient with subsiding erythema nodosum is regularly followed by an intense inflammatory reaction at the site of injection.

The development of this local reaction was followed in half of the subjects tested by a recrudescence of erythema nodosum in the areas recently affected.

The capacity of the involved extremities to develop erythema nodosum persisted for only a few weeks.

A possible relation between the induction of erythema nodosum and an antigen-antibody reaction is discussed.

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# VARIATIONS IN SERUM CALCIUM AND PHOSPHORUS DURING PREGNANCY. III. THE EFFECT ON THE FETAL CIRCULATION

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(Received for publication April 23, 1936)

In an earlier paper (1) we demonstrated that pregnancy causes a fall in both the calcium and inorganic phosphorus content of the maternal blood. This fall, averaging almost 5 per cent at its point of greatest effect, about six weeks before delivery, carries the calcium level below the lower limit of the range for normal non-pregnant women (2). No significance has been attached to this as regards the growing fetus since it has long been believed that the demands of the child were met at the expense of the mother. This belief was in part substantiated by evidence that the calcium and phosphorus content of the cord blood invariably runs higher than that of the maternal blood drawn at the same time (3). It seemed to us, however, that this question should not be closed without an investigation of the possibility of some correlation between the maternal and fetal circulations.

The subjects, young women ranging from 16 to 36 years of age, delivered in the hospital, were normal pregnant patients with one exception, a toxemia of pregnancy. The cord blood was obtained from the residual end of the cord immediately after the delivery of the baby. Venous blood was drawn from the mother at approximately the same time. The calcium and phosphorus determinations were made by the same methods, with the same precautions, used in our earlier investigations. The serum was removed from the clot as soon as practical, 30 to 60 minutes after drawing, and the determinations made on clear fresh aliquots.

The results are embodied in Tables I and II. In both, the cord findings are tabulated in columns according to short interval ranges of the corresponding maternal values. The averages of these tabulations indicate very clearly that the levels of the cord blood, although without an exception higher than the maternal, are dependent on those of the maternal circulation. Not only do the av-

TABLE I

*Distribution of the serum calcium findings of the cord blood on the basis of the corresponding determinations of venous calcium*  
(Figures given in mgm. Ca per 100 ml. serum)

8.0 to 8.49	8.5 to 8.99	9.0 to 9.49	9.5 to 9.99	10.0 to 10.49
10.53	10.55 10.70 10.30 10.55 10.55 9.94 10.35 9.00 11.00 10.27* 10.58 10.33 9.91 10.51	10.24 11.67 10.24 11.93 11.81 10.82 10.65 11.13 10.45 9.96 10.04 10.75 11.21 10.09 10.64 10.45 10.80 11.00 11.50 11.60 10.20 10.81 11.02 10.70 11.11	11.35 10.54 11.44 11.94 11.62 10.75 11.52 11.00 11.10 11.90 13.10 12.90 11.60 12.00 11.20 11.35	12.40 12.60 10.90 11.90 11.90 12.50 11.10 11.70
Average	10.32	10.83	11.58	11.86
Range	9.00 to 11.00	9.96 to 11.93	10.54 to 13.10	10.90 to 12.50

\* A toxemia patient, blood pressure 188/128, calcium findings not unusual.

erages show a definite rise as the maternal values increase, but the maximum and minimum findings for each column, with one exception in each table, show the same rise.

No effort has been made so far to establish a normal range for either calcium or phosphorus in the fetal circulation, nor have definite minimum requirements been established for proper growth and development. We have only shown that the calcium and phosphorus findings in cord blood tend quite definitely to vary with the findings of the corresponding maternal blood. But, when the blood calcium of the average normal pregnant

TABLE II

*Distribution of the serum phosphorus findings of cord blood on the basis of the corresponding determinations of venous phosphorus*  
(Figures given in mgm. P per 100 ml. serum)

2.0 to 2.99	3.0 to 3.99	4.0 to 4.99	5.0 to 5.99	6.0 to 6.99
6.50	5.20	5.10	7.30	7.60
5.00	5.50	6.50	6.10	7.10
3.73	3.80	5.60	7.10	6.95
5.27	5.40	5.90	6.30	
	4.80	5.65	7.20	
	5.48	6.05	5.70	
	6.52	6.63	5.60	
	5.13	5.53	8.97	
	5.00	6.58	5.88	
	5.56	5.88	6.98	
	5.51	5.70	7.07	
	4.35	5.88	6.90*	
	3.94	6.79		
	5.28	5.72		
	5.62	5.85		
	5.83	6.99		
	4.17	5.63		
	5.27	6.51		
	5.88	6.70		
		6.72		
		6.47		
		7.18		
Average 5.12	5.17	6.15	6.75	7.22
Range 3.73 to 6.50	3.80 to 6.52	5.10 to 7.18	5.60 to 8.97	6.95 to 7.60

\* A toxemia patient, blood pressure 188/128, phosphorus findings not unusual.

woman is depressed below the lower limits of the normal range, it is not hard to conclude that the cord values found in such instances are below

the levels optimal for the fetus, and that impairment of bone and tooth development may thus result in utero. The value of maintaining the calcium and phosphorus levels of the blood during pregnancy is, therefore, possibly more important to the development of the fetus than to the health of the patient herself.

#### CONCLUSIONS

It has been demonstrated that, although higher, the calcium and phosphorus levels of cord blood are dependent on the levels of the maternal blood.

These findings suggest that the lower values of cord blood, resulting from the lowered maternal values usually found in pregnancy, may represent an inadequate availability of calcium and phosphorus for fetal metabolism.

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# VARIATIONS IN SERUM CALCIUM AND PHOSPHORUS DURING PREGNANCY. IV. EFFECT ON THE BODY STORES AS SHOWN BY THE ASH OF RATS

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The evidence of a fall in the calcium and inorganic phosphorus content of the serum of normal pregnant women (1) served to confirm the general belief that the demands of pregnancy tended to exhaust the calcium and phosphorus resources of the body. A fall in the serum content, however, does not indicate whether the withdrawal is merely from the circulating medium or whether there is a corresponding depletion of the body stores. We have shown that, contrary to common belief, the teeth are not a source of supply (2). This is entirely logical, since there are no means by which the body could absorb calcium from them for use elsewhere. It is quite possible, on the other hand, for large amounts of mineral to be removed from the bones to meet an increased demand (3). Such a loss would be revealed by an examination of the calcium and phosphorus content of the body ash.

This study has been carried out on the rat, an animal small enough to be ashed with convenience, and with dietary habits that can be compared to those of man. The animals, of a good healthy stock, were maintained on a diet adequate in all respects, with no curtailment of either minerals or vitamins. Those sacrificed were anesthetized, bled to death from the carotid artery, and disemboweled. If pregnant, the uterus and young were removed together for separate examination. The blood was allowed to clot, the serum removed for analysis, and the clot returned to the carcass. The body was then weighed, dried for several days in an oven at 100 to 125° C., and finally ashed in a silica dish. The ash was dissolved by warming in 10 ml. concentrated hydrochloric acid, previously saturated with tartaric acid; the excess acid was then evaporated off on a steam bath, and the remainder dissolved in 500 ml. of distilled water. A small residue always settled out, but since tests showed this did not

interfere with the determinations, it was, for convenience, allowed to remain undisturbed on the bottom of the flask. Since this was a study of the body resources, particular emphasis was laid on ashing the entire carcass, with the sole exception of the intestinal tract, which would contain unabsorbed minerals from the food.

Calcium determinations were made on small aliquots of the ash solution by the same method as used for blood in our previous work (1, 4, 5). This method was satisfactorily checked at that time against gravimetric determinations. Multiple analyses on the same amounts of solution, the use of different amounts, and the recovery of added calcium all assured accuracy with this procedure. It was also used for the serum calcium, with the exception that the serum was weighed instead of measured by volume. This was more accurate in the instances where less than 2 ml. of serum was available, and also makes the result directly comparable with those of the ash.

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Four different conditions were studied, non-pregnant controls, a group 19 to 21 days pregnant, a newly delivered group, and one taken at the time of weaning. The controls were unbred animals of approximately the same age and weight as those having their first litter. The pregnant group were animals carrying their first or second litters. They were sacrificed just before delivery, when the young removed showed definite movement inside the uterus. The newly delivered group were sacrificed within 24 hours of the time their litters were dropped, but after the young had



TABLE II

*Distribution of the serum phosphorus findings of cord blood on the basis of the corresponding determinations of venous phosphorus*  
(Figures given in mgm. P per 100 ml. serum)

2.0 to 2.99	3.0 to 3.99	4.0 to 4.99	5.0 to 5.99	6.0 to 6.99
6.50	5.20	5.10	7.30	7.60
5.00	5.50	6.50	6.10	7.10
3.73	3.80	5.60	7.10	6.95
5.27	5.40	5.90	6.30	
	4.80	5.65	7.20	
	5.48	6.05	5.70	
	6.52	6.63	5.60	
	5.13	5.53	8.97	
	5.00	6.58	5.88	
	5.56	5.88	6.98	
	5.51	5.70	7.07	
	4.35	5.88	6.90*	
	3.94	6.79		
	5.28	5.72		
	5.62	5.85		
	5.83	6.99		
	4.17	5.63		
	5.27	6.51		
	5.88	6.70		
		6.72		
		6.47		
		7.18		
Average 5.12	5.17	6.15	6.75	7.22
Range 3.73 to 6.50	3.80 to 6.52	5.10 to 7.18	5.60 to 8.97	6.95 to 7.60

\* A toxemia patient, blood pressure 188/128, phosphorus findings not unusual.

woman is depressed below the lower limits of the normal range, it is not hard to conclude that the cord values found in such instances are below

the levels optimal for the fetus, and that impairment of bone and tooth development may thus result in utero. The value of maintaining the calcium and phosphorus levels of the blood during pregnancy is, therefore, possibly more important to the development of the fetus than to the health of the patient herself.

#### CONCLUSIONS

It has been demonstrated that, although higher, the calcium and phosphorus levels of cord blood are dependent on the levels of the maternal blood.

These findings suggest that the lower values of cord blood, resulting from the lowered maternal values usually found in pregnancy, may represent an inadequate availability of calcium and phosphorus for fetal metabolism.

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# VARIATIONS IN SERUM CALCIUM AND PHOSPHORUS DURING PREGNANCY. IV. EFFECT ON THE BODY STORES AS SHOWN BY THE ASH OF RATS

By J. W. MULL

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(Received for publication May 18, 1936)

The evidence of a fall in the calcium and inorganic phosphorus content of the serum of normal pregnant women (1) served to confirm the general belief that the demands of pregnancy tended to exhaust the calcium and phosphorus resources of the body. A fall in the serum content, however, does not indicate whether the withdrawal is merely from the circulating medium or whether there is a corresponding depletion of the body stores. We have shown that, contrary to common belief, the teeth are not a source of supply (2). This is entirely logical, since there are no means by which the body could absorb calcium from them for use elsewhere. It is quite possible, on the other hand, for large amounts of mineral to be removed from the bones to meet an increased demand (3). Such a loss would be revealed by an examination of the calcium and phosphorus content of the body ash.

This study has been carried out on the rat, an animal small enough to be ashed with convenience, and with dietary habits that can be compared to those of man. The animals, of a good healthy stock, were maintained on a diet adequate in all respects, with no curtailment of either minerals or vitamins. Those sacrificed were anesthetized, bled to death from the carotid artery, and disemboweled. If pregnant, the uterus and young were removed together for separate examination. The blood was allowed to clot, the serum removed for analysis, and the clot returned to the carcass. The body was then weighed, dried for several days in an oven at 100 to 125° C., and finally ashed in a silica dish. The ash was dissolved by warming in 10 ml. concentrated hydrochloric acid, previously saturated with tartaric acid; the excess acid was then evaporated off on a steam bath, and the remainder dissolved in 500 ml. of distilled water. A small residue always settled out, but since tests showed this did not

interfere with the determinations, it was, for convenience, allowed to remain undisturbed on the bottom of the flask. Since this was a study of the body resources, particular emphasis was laid on ashing the entire carcass, with the sole exception of the intestinal tract, which would contain unabsorbed minerals from the food.

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been cleaned up and the after-birth eaten by the mother. Those permitted to nurse their young were maintained until their offsprings, 21 to 24 days old, were able to eat and to take care of themselves, but had not ceased to nurse.

TABLE I

*The calcium and phosphorus content of adult female rats*

	Num- ber of rats	Aver- age weight	Cal- cium per 100 grams of serum	Cal- cium per gram of body weight	Phos- phorus per gram of body weight
		grams	mgm.	mgm.	mgm.
Normal non-pregnant . . . . .	10	136.4	10.04	11.54	7.32
19 to 21 days pregnant . . . . .	10	172.0	8.81	10.83	6.88
Newly delivered . . . . .	5	162.2	10.16	11.71	7.07
Nursed to weaning . . . . .	10	140.0	10.28	11.58	7.47
Additional controls . . . . .	8	116.1	10.28		

The results from the adult animals are summarized in Table I. The serum calcium findings of the pregnant group, averaging greater in weight than the controls, show a fall of 12.2 per cent in calcium, while both the newly delivered and nursing groups show a slight rise, less than 3 per cent. The calcium per gram of body weight, determined from the ash, of the pregnant group shows a 6.1 per cent fall from that of the normals, while the others show an insignificant rise, under 2 per cent. Similarly the pregnant animals show a 6 per cent loss in body phosphorus, while the newly delivered lost only 3.4 per cent, and the nursing group show a 2 per cent rise over the controls.

These serum calcium findings compare very well with those found in our study on pregnant women. In the human study the period of greatest depression occurred about six weeks before delivery, followed by a slight rise until delivery, then an abrupt return to normal. The 20th day of gestation in the rat is about the same proportion of the gestation period as the point six weeks prior to delivery is in the human. The recovery of the newly delivered animals, and the normal findings at the end of nursing, check very well with the available figures from the human series. With this close parallel in the serum calcium variations, it seems quite possible that the body stores, as represented by the ash, would show a similar parallel. This would indicate a real depression of

the calcium and phosphorus resources of the body during pregnancy, especially in those cases where the serum calcium and phosphorus levels were markedly lowered.

This conclusion is supported by the metabolic studies of Macy, Hunscher, Nims, and McCosh (7) who found calcium losses in two of their three human subjects about 10 weeks before delivery. We are, however, apparently in disagreement with the calcium and phosphorus studies of Goss and Schmidt (8), carried out on rats. They found positive calcium and phosphorus balances during pregnancy, and negative balances during lactation. Our body ash analyses indicate just the reverse. They are, nevertheless, in agreement both with the definite recoveries of the serum calcium and phosphorus in our human series following delivery, and the work reported by Donelson, Nims, Hunscher, and Macy (9) showing positive calcium and phosphorus balances possible during lactation. If, though, the lactation has been of long duration, the calcium balance may become negative.

TABLE II

*The calcium and phosphorus content of the young*

	Num- ber of litters	Aver- age num- ber per litter	Aver- age weight	Cal- cium per gram of body weight	Phos- phorus per gram of body weight
			grams	mgm.	mgm.
Unborn young . . . . .	11	6.63	30.6	1.016	1.888
New-born young . . . . .	6	6.66	34.9	2.195	2.698

The young removed from the pregnant animals were ashed in with the uterus and placenta, in silica dishes, but were dissolved in only 5 ml. of acid, and made to a final volume of 50 ml. The new-born were treated in the same way, but only the clean young themselves were ashed. The results are given in Table II. The marked difference in calcium and phosphorus between the two groups can be explained, in part at least, by the fact that a portion of the weight of the unborn group was made up of placenta and uterus. The difference between the new-born and the adults, however, are particularly striking, the calcium content of the adults averaging almost 5 times that of the young, with the phosphorus almost 3 times.

The calcium-phosphorus ratios are also worthy of note, those of the adults being roughly 11/7 while in the young it is 2.2/2.7 mgm. per 1 gram of body weight.

In view of the very low per cent of calcium and phosphorus in the body weight of the rat young, it is quite possible that considerable allowance should be made in attempting to project these findings to the conditions that pertain with the human mother. With the heavier human fetus containing 0.7 to 0.8 per cent calcium, or from 13 to 33 grams (10), the demands very probably are much greater in proportion, and an analysis of the maternal human ash would probably reveal a greater loss than the 6 per cent found in the rat.

#### CONCLUSIONS

Pregnant rats show a fall in serum calcium near the end of gestation averaging around 12 per cent from that of the non-pregnant controls. This was not found in the newly delivered rats or at the end of nursing.

The calcium and phosphorus per gram of body weight, as determined from the ash of the entire carcass, showed a 6 per cent loss near the end of gestation, from that of the non-pregnant controls. Newly delivered rats or those nursing their young to weaning did not show such a loss.

The fall in serum calcium and phosphorus is an indication of the depletion of the body stores, as determined from an analysis of the body ash. The analogous fall in the human during pregnancy can, therefore, also be taken as an indication of the depletion of the mineral stores of the body.

New-born rats showed a much lower calcium and phosphorus content per gram of body weight

than the adults, and an entirely different calcium-phosphorus ratio.

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# SUGARS AND GLYCOLYTIC ENZYMES OF SPINAL FLUID IN EPIDEMIC CEREBROSPINAL MENINGITIS

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In a preliminary note (1) the presence in spinal fluid of a fermentable substance giving the resorcinol (fructose) reaction was reported. The present paper contains the results of analyses made upon specimens from two patients with meningitis due to the meningococcus. The results upon these cases are given in detail for three reasons. First, repeated punctures were made, and it was therefore possible to confirm results by repeated studies; second, some of the specimens were so large that certain experiments could be carried out which can usually be done only upon mixtures of fluid from several patients; third, the nature of the fluid made possible a study, not only of the sugars themselves, but also of the effect of glycolytic enzymes upon them.

## METHODS

The methods used were the same as those previously employed. Protein was precipitated by zinc sulphate and sodium hydroxide in the proportions recommended by Somogyi (2). In working with spinal fluids the relatively low protein content made it possible to prepare filtrates containing a high proportion of the initial material; the proportions most commonly used were 1:1.2 and 1:1.4. Protein and the precipitated zinc were removed by centrifuging. Total reducing substances were determined in the supernatant fluid by the method of Folin and Wu (3, 4), modified, when the sugar content was very low, as recommended by Hubbard and Allison (5). Fructose was determined by the method of Roe (6). Because very little fructose was found in some specimens, a series of standards was prepared from solutions of the pure sugar ranging in concentration from 0.1 to 4.0 mgm. per 100 cc. These were analyzed simultaneously with the blood or spinal fluid filtrate, and the "unknown" compared with the standard nearest to it in tint. This comparison was made in a colorimeter when the apparent concentration was greater than 1.0 mgm. per 100 cc.; otherwise, the amount present was usually estimated by matching against a series of dilute standards in tubes of equal diameter. The accuracy of such determinations upon spinal fluid was approximately 0.1 to 0.2 mgm. per 100 cc. The accuracy upon blood and other fluids, which were diluted before analysis, was lower, and probably lay between 0.5 and 1.0 mgm. per 100 cc. Because glucose gave a slight color with the

resorcinol reagent which closely resembled that given by fructose (1), correction tables based upon the analysis of pure glucose from the Bureau of Standards and of mixtures of this glucose with pure fructose were prepared. Since the "fructose" and other non-glucose reducing compounds contributed only a small fraction of the total reducing power of these fluids, the results of the Folin and Wu determinations were used as a basis for applying the appropriate corrections. These ranged from 0.0 to 0.4 mgm. per 100 cc. for the specimens of spinal fluid. The corrections of determinations upon blood plasma and serum were larger and amounted to as much as 2.0 mgm. per 100 cc. in some instances.

Both of the cases of meningitis upon which this report is based were typical. The diagnosis rested in each instance upon the presence of meningococci in smears and cultures prepared from the spinal fluid when the patients were first seen. Thereafter, the organisms were usually not found in the smears, but could often be recovered by an appropriate culture method.<sup>1</sup> Both patients were treated by repeated injections of anti-meningococcus serum into the spinal canal. Both eventually responded to the treatment in a satisfactory manner, but improvement was less rapid in Case 1 than in the second patient studied and was interrupted by two exacerbations of his condition. Brief reports of these cases are given below.

*Case 1* was a white man, 21 years old, who was admitted October 31, 1935, in a semi-comatose condition. His temperature was 104° F. Marked rigidity of the neck and a positive Kernig's sign were present. Knee jerks were absent. He had had headache, nausea and vomiting for 24 hours preceding admission. The spinal fluid was cloudy and under a pressure of 250 mm. of water. The protein concentration was 0.49 per cent. The cell count was 2000 per c.mm., with polymorphonuclear leukocytes predominating. Diplococci with morphological and cultural characteristics of meningococci were found in a smear and recovered by culturing the fluid. Twenty cubic centimeters of New York State anti-

<sup>1</sup> The microscopic and cultural examinations were carried out in cooperation with the bacteriological laboratory of the hospital.

meningococcus serum were given intraspinally at once. The treatment used consisted of repeated spinal punctures and daily injections of from 20 to 30 cc. of specific serum into the spinal canal. In all, 15 punctures were made and 270 cc. of the serum given. The cell count remained very high until the 9th of November, when the cells numbered 280 per c.mm. At that time a negative culture was first obtained, and the temperature fell to normal by lysis. On November 14th the temperature was again high, the spinal fluid contained 1900 leukocytes per c.mm., and the culture was again positive. No positive cultures were obtained after this date, but the temperature was frequently very high and the cell counts in different specimens of spinal fluid varied markedly, for 4400 leukocytes per c.mm. were found on November 16th, 825 on the 18th, 88 on the 20th, and 300 on the 22nd. During this period, either the type or the staining characteristics of the leukocytes varied quite markedly, for a predominance of eosinophilic cells was reported in several of the specimens. On the 5th of December, after the clinical condition of the patient had become practically normal, a specimen of spinal fluid containing 30 cells per c.mm. was obtained. These cells were classified as lymphocytes.

*Case 2.* The second patient was a girl 16 years old. She was admitted on December 17, 1935, giving a history of a cold which had lasted for 10 days. She had developed chilliness, a severe headache, and stiffness of the neck four days before she entered the hospital, and nausea, vomiting and a purpuric eruption on December 16th. She became delirious on the day of admission. She showed irritability, a stiff neck, a positive Kernig's sign, and a bilateral Babinski's sign. Her temperature was 105° F. Spinal fluid obtained on admission contained 6600 cells per c.mm. and 0.31 per cent protein. Organisms resembling meningococci were found in the smear and on culture. Twenty cubic centimeters of therapeutic serum were injected into the spinal canal at once. Eleven spinal punctures were done during the succeeding nine days, and an injection of 20 cc. of the serum was given at the time of each puncture. Positive cultures of meningococci were obtained daily, and the cell count remained high until the 21st, when a spinal fluid containing 170 cells per c.mm. was sterile on culture. Thereafter, the patient's condition improved rapidly, and a sterile specimen of fluid containing only 11 cells was drawn on December 28th. The cells were at all times considered to be polymorphonuclear leukocytes.

Besides determinations of the sugars in the different fluids made as soon as possible after they were obtained, the effect of incubation upon their apparent glucose and fructose content was studied. For this purpose fluids drawn with precautions to avoid bacteriological contamination were placed in an incubator at 37.5° C. and the analyses repeated after various periods. In some instances, control specimens were placed on ice and analyzed simultaneously with the incubated ones to reduce technical errors. The results of these determinations are given in Table I.

TABLE I  
*Sugars in spinal fluid before and after incubation †*

Case	Material	Date	Incubated	Before incubation		After incubation	
				"Glucose"	"Fructose"	"Glucose"	"Fructose"
		1935	hours	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
1	Spinal fluid	Nov. 4	24	6.7	1.2	2.0	1.2
		Nov. 9	48	6.6	0.8	3.6	0.9
		Nov. 11	24	55.0	3.5	53.0	3.0
		Nov. 14	48	19.3	0.8	3.2	0.9
		Nov. 15	72	42.8	0.9	2.8	0.8
		Nov. 16	48	10.9*	0.6*	3.4	0.4
		Nov. 18		32.2	0.8		
		Nov. 19	24	31.5	0.2	26.5	0.3
		Nov. 20	24	17.0*	0.4*	18.4	0.2
		Nov. 21	24	10.3	0.3	2.4	0.3
		Nov. 30		53.0	0.8		
	Blood	Nov. 4		96.6	0.5		
		Nov. 14		111.0	0.0		
		Nov. 16		103.0	0.2		
	Plasma	Nov. 16		113.0	0.1		
2	Spinal fluid	Dec. 16		1.2	0.2		
		Dec. 17		3.3	0.5		
		Dec. 17	24	9.1	0.7	2.5	0.6
		Dec. 18		6.1	0.7		
		Dec. 19	24	16.3*	1.2*	2.7	1.0
		Dec. 19	24	12.5*	0.7*	2.8	0.7
		Dec. 20	24	27.0	0.7	4.2	0.8
		Dec. 21		41.3	1.0		
		Dec. 23	24	45.0	1.4	44.4	1.0
		Dec. 28		55.7	1.5	43.4†	1.2†
	Blood	Dec. 17		135.0	0.3		
		Dec. 20		100.0	0.0		
		Dec. 28		103.0	0.7		
	Therapeutic serum			20.0	0.0		

\* The control specimen was kept on ice and analyzed simultaneously with the incubated one.

† Many red blood cells were present in this specimen of spinal fluid.

‡ Under "glucose" is given the total reducing substances in terms of glucose, and under "fructose" a quantitative measure of the intensity of the resorcinol reaction as fructose. Results of determinations of fructose have been corrected for the slight resorcinol reaction given by pure solutions of glucose of the same concentrations as those present in the solutions analyzed.

The results show that both glucose and fructose were apparently present in most of the specimens, although the amounts were low in some instances. A low glucose content of fluid from patients with meningitis due to the meningococcus has been repeatedly observed; in this respect epidemic meningitis does not differ from other similar conditions. The fructose concentration of the fluid in these two cases was also low, for most of the results were between 90 and 50 per cent lower than those found in most normal fluids. Such a finding was not unexpected, for some parallelism between the amounts of glucose and fructose in normal spinal fluids was found by Hubbard and Garbutt (1); furthermore, those authors reported

that no fructose was found in one specimen of a fluid from which glucose was absent. Hubbard and Garbutt suggested, as a possible explanation for the presence of fructose in spinal fluid, that the sugar might be formed from glucose in the spinal canal by a molecular rearrangement similar to that which glucose undergoes in alkaline solutions. The finding of low values for both sugars in the cases of meningitis reported here may perhaps be interpreted as evidence in favor of such an hypothesis, but there are so many other factors which might affect the finding, such as the abnormal physical and chemical nature of the spinal fluid, the presence of a pathological process in the spinal canal, the introduction of the serum used in the treatment, etc., that it seems dangerous to emphasize the support which these observations seem to lend to the theory previously suggested.

Table I shows that, after incubation, the concentrations of reducing substances were usually lower than were those found when the control specimens were analyzed. The eleven fluids which showed this difference were cloudy and had a high protein content,<sup>2</sup> the three which did not were much more nearly normal in appearance and contained reducing substances in amounts approximating those found in normal fluids. Two of these specimens were taken from the first patient during remissions; the other was obtained from the second patient when recovery was almost complete.

Although incubation brought about a marked decrease in the reducing power of most of the specimens studied, it caused little or no alteration in their "fructose" content. The average decrease in concentration was 0.06 mgm. per 100 cc. In only 2 of the 14 specimens did the concentration fall by more than 0.2 mgm. per 100 cc. Both of these were fluids of approximately normal composition which did not show a significant drop in reducing power after incubation.

<sup>2</sup> The results of cell counts made upon the various fluids ranged from 100 to 6600 per c.mm. These counts were made as soon as possible after the specimens were taken. Most of the fluids contained fibrin, and many of the cells became entangled in the web which formed quite promptly. Figures obtained in the counts made before the clot formed, therefore, bore little relationship to the concentration of cells suspended in the incubated fluid. The results have, therefore, been omitted in preparing the table.

The fall in the total reducing power observed must have been due largely to destruction of glucose. Results of the order of magnitude of those found can be explained in no other way. Since this destruction of glucose was not accompanied by a significant change in the intensity of the corrected "fructose" reaction, the positive resorcinol test must have been caused by some substance other than glucose.

Figures showing the apparent fructose content of the blood and plasma of the patients and of the serum used in the treatment are also shown in Table I. It is evident that the amount of this compound present was low in each instance. The significance of the figures is questionable for two reasons. The color measured in the dilute filtrates was very faint, and corresponded to that given by fructose solutions containing between 0.1 and 0.2 mgm. of the sugar in 100 cc. In each instance the color given by solutions of pure glucose similar in concentration to those contained in the dilute filtrates would account for between 75 and 100 per cent of all the color found. It is evident, therefore, that no quantitative significance can properly be attached to the figures, and it seems questionable whether the results definitely prove the presence of any fructose or fructose-like substance in the blood, serum, and plasma analyzed.

Various experiments were carried out to determine the mechanism by which incubation brought about a decrease in the reducing power of these spinal fluid specimens. When specimens were kept on ice for 24 hours no destruction of "glucose" or "fructose" could be demonstrated. When cells were removed by centrifuging and the supernatant fluid incubated, there was also no reduction in the concentration of either of the "sugars." The results of these experiments agree with the commonly accepted theory (7, 8), that much of the glycolytic enzyme in spinal fluid is contained in the cells and bacteria.

The results of the following experiment further confirm the impression that the glycolytic enzyme was contained in the cells, and show further that the sugars in fluid from meningococcic meningitis were probably not different from those found in normal fluid. Five cubic centimeters of a fluid rich in cellular elements, which was obtained from



the second patient, were centrifuged under sterile precautions. The supernatant fluid was discarded and replaced by 5 cc. of a "normal" fluid obtained during a diagnostic encephalogram. The cells were then suspended in the normal fluid, and the tube and its contents incubated at 37.5° C. for 24 hours. A similar suspension of cells in sterile 0.85 per cent sodium chloride was treated in a similar manner. The next day the cells were removed by centrifuging, and the total reducing power and "fructose" content of the supernatant fluids determined. Analyses of a specimen of the normal fluid which had been incubated without the addition of cells and of another which had been kept on ice 24 hours were made simultaneously. The reducing power of the normal fluid was almost completely destroyed in the presence of the cells (a drop in concentration of 45 mgm. of "glucose" per 100 cc. from an initial value of 50 mgm. was observed), but the "fructose" content fell only 0.4 mgm. from an initial value of 2.3 mgm. Neither "glucose" nor "fructose" was found in the salt solution in which the cells had been incubated, nor was there any significant difference between the amounts of the two sugars in the specimen incubated without the addition of cells, the fluid kept on ice, and the control specimen analyzed soon after the fluid was drawn.

It seemed desirable to determine whether pure fructose could be added to a spinal fluid rich in cells and be recovered after incubation. The sugar was dissolved in sterile 0.85 per cent sodium chloride solution and added to such a specimen of spinal fluid obtained from Case 1 to give an additional fructose concentration of 0.5 mgm. per 100 cc. An equivalent amount of the saline was added to a duplicate sample of the same spinal fluid. Both specimens were incubated at 37.5° C. for 48 hours. Ninety-five per cent of the 40 mgm. of "glucose" contained in 100 cc. of these specimens was destroyed during this incubation. The added fructose was recovered quantitatively.

#### DISCUSSION

It has been shown that the enzymes in the cellular elements in these fluids caused certain changes in the sugars which were present. The enzymes may have been contained either in the organisms or the leukocytes. Smears were prepared from fresh samples of all of the specimens

studied and organisms demonstrated in only two of them. Smears were also made from centrifuged material after incubation and organisms recovered in only one instance. Such findings are common in material from active cases of meningococcic meningitis (9, 10), and make it seem probable that the leukocytes contained the active agent. However, when suitable culture methods were used, meningococci could be demonstrated in many of the fluids, and Mader (11) has shown that glucose is sometimes destroyed when that organism is added to spinal fluid. Thirteen specimens of "normal" spinal fluid, including two obtained from Case 1 after recovery, were, therefore, heavily inoculated with meningococci and incubated for periods of 24 to 96 hours under conditions identical with those used in the experiments reported above. In 8 of these specimens there was no growth of organisms. In 5, a rather scanty growth occurred, and in only 2 of the whole series was there a measurable destruction of sugar. In neither of these experiments did the changes in concentration of sugar resemble those shown in Table I, for, in one instance, both glucose and fructose were apparently completely destroyed and in the other a destruction of 50 per cent of the glucose and of 90 per cent of the fructose was found. These experiments make it seem probable that the changes shown in Table I were not brought about by organisms in the spinal fluid studied, but by enzymes present in the leukocytes.

In the previous communication (1) it was reported that the substance giving the resorcinol (fructose) reaction was destroyed by short incubation with large amounts of yeast. This observation was confirmed upon material from each of these two cases. The method used was similar to those described by Hiller, Linder and Van Slyke (12), Somogyi (13), and Benedict (14). Large amounts (0.25 cc. to 2.5 cc.) of centrifuged, washed yeast cells were introduced into 5 cc. portions of the fresh and the incubated spinal fluid. The mixtures of the fluid and yeast were then placed in the incubator at 37.5° C. for 15 to 20 minutes. No "fructose" could be demonstrated in specimens which had been treated in this way, and the reducing power was markedly lower than it had been before the yeast was added.

The apparent glucose content was between 1 and 2 mgm. per 100 cc.<sup>3</sup> Values of this order of magnitude have been repeatedly obtained in studies of spinal fluid made in this laboratory. They apparently represent some non-sugar reducing substance which is quite regularly present in spinal fluid.

To confirm these results with yeast and to exclude the rather remote possibility that the resorcinol reaction in spinal fluid was due to sucrose, a strain of bacterium coli, which did not attack that sugar but which did destroy glucose and fructose, was used. A specimen of spinal fluid from Case 1 was heavily inoculated with these organisms and incubated for 24 hours. After this period the results of a resorcinol test were completely negative. The apparent glucose content was 1.2 mgm. per 100 cc. These results with yeast and bacterium coli can be adequately explained if the specimens contained glucose, fructose (or some compound closely resembling fructose), and a small amount of non-fermentable reducing substance. It seems very difficult to interpret them in any other way.

#### SUMMARY

In specimens of spinal fluid from two patients with meningococcic meningitis a substance giving a typical positive test for fructose by Roe's (6) method was present. The biological properties of this substance were apparently identical with those of fructose. By incubating specimens of this fluid the concentration of the reducing substances was markedly diminished, while the concentration of the "fructose" was little altered.

<sup>3</sup>In two instances the incubation with cells and the treatment with yeast were carried out upon duplicate specimens of spinal fluid. The average of the values after 15 minutes incubation with yeast was 1.5 mgm. "glucose" per 100 cc., while the corresponding value after 24 hours incubation with cells was 3.9 mgm. The accuracy of the determinations of such small concentrations of "sugar" is, of course, low, but the difference between the figures is probably significant. The difference seems too great to be wholly attributed to "fructose," which was destroyed by the yeast but was wholly unaffected by the cells, for an average concentration of only 0.9 mgm. of that sugar per 100 cc. of fluid was found in these specimens. The difference was probably due in part to "fructose" and in part to glucose, which apparently was not wholly destroyed by the incubation with the cells.

The glycolytic enzyme affecting the change seemed to be present in the leukocytes, for the change was not observed when specimens were freed from cells before incubation, nor was it found in spinal fluids which were inoculated with meningococci and incubated under comparable conditions.

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# ENCEPHALITIS IN NORTH CHINA. RESULTS OBTAINED WITH NEUTRALIZATION TESTS

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The work of Muckenfuss, Armstrong and McCordock (1) and of Webster and Fite (2) has established the fact that the type of encephalitis prevalent in St. Louis during the summer of 1933, was due to a filtrable virus. The latter succeeded in transmitting the virus to mice. They showed by means of protection tests (3) that neutralizing antibodies were present in the sera of convalescent encephalitic patients. In this way Webster and Fite demonstrated that the epidemic in St. Louis was due to a single virus which differed from other viruses previously described (4). Furthermore the experiments of Webster and Fite, and Wooley and Armstrong (5) indicate that the St. Louis type of encephalitis is etiologically distinct from Economo's encephalitis. The results of neutralization tests suggest that the first appearance in the United States of the virus of the St. Louis type occurred in 1932, in Paris, Illinois.

Clinically, the St. Louis type of encephalitis closely resembles the so-called "B" type of Japanese encephalitis, first differentiated in the epidemic occurring in the vicinity of the Inland Sea, Japan, in 1924 (6). However, Webster and his coworkers (4) failed to demonstrate neutralizing antibodies in the sera of Japanese patients who had recovered from the "B" type of encephalitis either in 1924 or 1933.

In view of the fact that the results of Webster and his colleagues seemed to indicate that the St. Louis type of encephalitis differed from that prevalent in Japan, it was of interest to determine whether two cases of encephalitis observed in Peiping, China, belong to the Japanese or the St. Louis type. The majority of the cases of the St. Louis type and of the "B" type in Japan seem to run a short acute course. Recovery, if it takes place, is usually complete. The two cases to be reported here have to date failed to recover completely, and one of the patients had an unusually prolonged and severe course. It seems of

interest, therefore, to give brief summaries of the histories of these cases. Both patients were American women; one contracted the disease in North China during the summer of 1934, the other in Japan during the summer of 1935. (The second patient became acutely ill on the boat on her way from Japan to North China, one day after sailing from Kobe.)

## Case 1

Mrs. W., a 27-year old American married woman, came to China for the first time in the fall of 1933. She lived in Peiping until June 15, 1934, when she went to a seaside resort, in the province of Hopei, about 200 miles north of Peiping. She remained well until July 28, 1934. She was 7½ months pregnant at this time, but had no abnormal symptoms. On July 28th she developed a discrete erythematous rash which faded on pressure and which involved mainly the trunk. She was seen by a doctor who thought she had an allergic reaction probably due to eating crabs. On the following day, July 29th, the rash was fading, but she had an extremely intense headache, and seemed sick. On July 30th the headache had improved slightly, but she was somewhat irrational. The temperature fluctuated between 37° and 39.2° C. She never vomited. Her neck was slightly stiff on July 30th, but Kernig's sign was not definitely positive. On July 31st, the day of admission to the hospital in Peiping, the patient was irrational, the neck was less stiff, and the headache still present, though less intense than it had been on July 29th. No similar disease was noted in other members of the community.

*Physical examination.* The patient was a well-nourished and well-developed pregnant woman who was irrational. The temperature on admission was normal but rose to 38.4° C. within the first 12 hours. This was the highest point the temperature reached throughout the course of the illness. The reflexes were all hyperactive; no definite Kernig's sign was elicited.

*Laboratory findings.* The white cell count was 22,000 with a differential showing 94 per cent neutrophilic polymorphonuclears. The spinal fluid was under normal pressure, clear and colorless. The fluid contained 460 cells, 63 per cent of which were neutrophilic polymorphonuclears. Tests for increased globulin content were positive. A second lumbar puncture was done on the following day, August 1st; the total cell count was 383 with 49 per cent polymorphonuclears. The sugar content of the spinal fluid was found to be 93 mgm. per

cent, while the blood sugar was 118 mgm. per cent. The chloride content of the spinal fluid was 717 mgm. per cent.

*Course.* The patient was restless and irrational the second day after admission. On the third day she became drowsy and was irritable when roused. On questioning she complained of diplopia. Papilledema was present on both sides, slightly more marked on the right. Retention of urine developed, and the patient had to be catheterized twice. A coarse intention tremor of both hands was noted on August 2nd which persisted for several weeks. The temperature became normal 48 hours after admission and showed no further elevation. Her mental condition improved very slowly. She became less drowsy and answered questions more readily. Perseveration was fairly marked, and there was also some tendency to confabulate. It was found that she was completely disoriented as to time and place and that her recent memory was greatly impaired. She did not remember that she was married, or realize that she was pregnant. She was emotionally unstable, laughing and crying without cause.

The patient remained in the hospital for almost 2 months. She went through a normal labor and was delivered spontaneously. The baby showed no abnormalities. There was very little change in the patient's mental condition following delivery. She did not remember going through labor, and did not realize that she had a baby. She continued to be emotionally upset. She was sure that she was going to die, and at intervals became very much frightened. She was discharged 19 days after delivery on September 26, 1934. At that time her physical condition was excellent, the tremor of her hands had subsided entirely. Her mental condition, however, was still extremely unsatisfactory.

*Subsequent course.* The patient moved away from Peiping, but it has been possible to keep in touch with her from time to time. According to her husband, who is a physician, her personality has undergone a marked change. She is now able to look after the baby and shows some interest in her immediate household. She has, however, become suspicious of her husband and takes little part in the community where she lives. She refused to return to the hospital for re-examination when she visited Peiping 8 months after discharge. It has been possible to obtain blood from her for neutralization tests on one occasion on January 4, 1936, about 1½ years after her recovery. The results of these tests will be reported in conjunction with the other cases.

## Case 2

*Onset:* Mrs. G., a 32-year old American married woman, had been living in Tokyo, Japan, for 3 years prior to her coming to China for a visit in August 1935. She took the boat at Kobe on August 19, 1935. On August 20th she developed a temperature of 40.3° C. and complained of numbness of one hand. She had 3 severe vomiting spells. On August 21st her temperature was 38.8° C. and she complained of an intense headache. On

August 22nd the patient still complained of severe headache, and lapses of memory were noted. She developed shooting pains in the muscles of the right leg and became irrational. On August 23rd her temperature was 40° C.; she was unaware of her transfer from boat to train and did not recognize the names of friends.

*Course in hospital: August 23, 1935 to December 18, 1935.* The patient appeared critically ill, her temperature was 40.1° C. She was able to talk, but was irrational. Half an hour after admission the patient had a generalized convulsion lasting several minutes. On August 24th the eyegrounds showed mild optic neuritis. The most striking symptoms on admission were drowsiness and mental confusion. Signs of meningeal irritation only developed 48 hours later, and at the same time an increasing spasticity of the extremities was noted.

*Laboratory findings.* The white cell count was 16,500 with a differential showing 86 per cent neutrophilic polymorphonuclears. The spinal fluid was under increased pressure (375 mm. of water); clear and colorless; total cell count 228, with 75 per cent neutrophilic polymorphonuclears; tests for increased globulin content were positive; sugar 80 mgm. per cent.

The temperature fell from 40° C. to 38° C. in the course of 4 days. The patient's drowsiness increased, and she was no longer able to speak or move. Her attention could be aroused momentarily, and she would occasionally try unsuccessfully to obey commands to open her mouth and stick out her tongue. Her deep reflexes were all hyperactive, and no differences were noted on the two sides. The abdominal reflexes were absent. Marked cogwheel rigidity of all four extremities was present. No impairment of sensation could be determined. The Babinski sign was positive on the left, but only an equivocal response could be obtained on the right. She developed slight ptosis of the left eyelid which lasted for several weeks, and then cleared up. Rhythmic movements of the tongue and lower jaw were present for a short time. A coarse tremor of both hands, more marked on the left, persisted during most of the stay in the hospital and then diminished gradually.

The patient had an irregular fever, the temperature ranging between 38° and 39° C. for a period of 7 weeks. She had numerous furuncles during this time, but it was thought that the fever was related to the encephalitic process rather than to the condition of her skin.

The patient remained extremely drowsy for two months and had to be fed by nasal tube during most of that time. The rigidity of her extremities, particularly the lower, was marked. She held her legs persistently flexed, so that it was feared that she might develop permanent contractures. She was given atropine in increasing amounts until a maximum of 30 drops of 1-100 atrophine sulphate was reached. The spasticity decreased gradually and did not return when the atropine was stopped. During the time that the patient was receiving atropine, there was some dryness of the throat, but her pulse rate showed no significant elevation.

As the spasticity cleared up, the patient complained of

numbness of the 4th and 5th fingers of the left hand which persisted for several weeks and then disappeared. Slight hypo-esthesia over the dorsum of the 4th and 5th fingers and also over the dorsum of the left foot and the lateral aspect of the left leg was noted for a short period. With the decrease of the spasticity of the left leg, it became apparent that the peroneal and tibial muscles of the left leg, and the intrinsic muscles of the left foot were paralyzed. There was also weakness of the left gastrocnemius and left quadriceps. The patient's left foot was put in a splint on November 8, 1935, and she received daily massage and light treatments. The anterior tibial group of muscles showed slight atrophy. The left patellar reflex became hyperactive as compared to the right, and the left Achille's reflex hypoactive.

*Mental status.* The patient's mental condition was very poor when the fever subsided and she was able to talk. She was completely disorientated as to time and place and confabulated. Her recent and distant memory were both impaired. There was a striking improvement in her mental state before she was discharged. Her distant memory seemed normal, but her memory for recent events remained unreliable. She showed little insight into her illness or her financial situation.

On her discharge December 18, 1935, she returned to America and her family report that she seems to be improving. The muscular power of her left leg is somewhat better, and she is now able to walk without crutches. Except for inability to concentrate and lack of initiative, no striking personality changes have been observed by her relatives. Her memory is improving slightly.

Two samples of her blood were obtained for neutralization tests: one early in the course of the disease in September 1935, the other during convalescence in December 1935.

In addition to these cases which appeared to be clinically typical of epidemic encephalitis, it seems of interest to report a third case which was thought to be possibly vaccine encephalitis. In spite of the fact that encephalitis of both the "A" and "B" type is common in Japan, cases of encephalitis of any kind are not common in China. Although vaccination is generally practised throughout China, vaccine encephalitis is practically unknown. Only one case of possible encephalitis following vaccination has been reported, which occurred in a Chinese infant, aged  $1\frac{1}{2}$  months (7). The findings in this case, however, were not typical, since the vaccination failed to take. As far as we have been able to determine, no case of vaccine encephalitis has ever occurred in the absence of a successful vaccination. Although it is impossible to prove the diagnosis of vaccine encephalitis in a single case if

recovery takes place, it was thought of interest to exclude epidemic encephalitis as a possible cause by neutralization tests.

### Case 3

The patient, a Chinese girl of 8, was admitted on September 17, 1935, for coma of 4 hours' duration. She had been vaccinated for the third time on September 7, 1935, with a definite "take." The first vaccination was done shortly after birth. A very small faint scar was visible on the leg. The second vaccination done some-time later, had failed to take. At the time of admission, she showed a good take with a scar 1 cm. in diameter.

The patient had been well until September 16th, 9 days following vaccination, when she complained of headache. She vomited once on September 16th and again the morning of September 17th. She was given aspirin, but it failed to relieve the headache. At 11 o'clock on September 17th, the patient was found to be comatose.

*Physical examination.* On admission, the patient was stuporous and could not be aroused. She was restless, throwing herself from side to side, with teeth clenched. Her temperature was  $37.8^{\circ}$  C. on admission and rose to  $39^{\circ}$  C. within 8 hours. Her extremities were flaccid, the deep reflexes active and equal. The abdominal reflexes were absent. The Babinski sign was positive on both sides. No impairment of sensation was noted. Her neurological signs were found to vary considerably from time to time. Her eyegrounds were normal.

*Laboratory findings.* The white cell count was 11,000 with a differential showing 89 per cent neutrophilic polymorphonuclears. The pressure of the spinal fluid was normal. The fluid was clear and colorless and contained 67 white blood cells, 88 per cent of which were lymphocytes. The globulin content of the spinal fluid was slightly increased. The sugar content of the spinal fluid was 91 mgm. and chlorides 699 mgm. per cent. Her fasting blood sugar on the same day was 154 mgm. per cent.

*Course.* The patient was given glucose intravenously and fluids by nasal tube. Lumbar punctures were performed daily for the first 3 days. The patient regained consciousness at the end of 48 hours, and was talking normally on the third day. Her recovery was rapid and complete. Blood for neutralization tests was obtained shortly after recovery and again in March 1936, 5 months after discharge.

### *Attempts to demonstrate a virus in the spinal fluid*

The spinal fluid was inoculated intracerebrally into 2 rabbits and into 2 mice. All the animals remained well. One rabbit was killed on the 10th day, the brain was removed with sterile precautions and emulsified. The emulsion was injected intracerebrally into another rabbit. This rabbit had received several applications of coal tar over

a small area of skin. The brain emulsion was injected intradermally into normal and tarred skin. No reaction took place and the rabbit remained well. The patient's spinal fluid was also rubbed into the scarified cornea and injected intradermally and by scarification into the tarred and normal skin of another rabbit. This animal also failed to show a reaction of any kind.

#### Neutralization tests

The virus used for neutralization tests was obtained from 2 sources.

*Japanese virus.* Dr. Hashimoto of St. Luke's International Clinic succeeded in isolating a virus, transmissible to mice, from a fatal case of encephalitis in Tokyo in September 1935. He was kind enough to send us two infected mouse brains. We were able to transmit the virus from mouse to mouse without difficulty by intracerebral inoculation. The virus was active in a dilution of  $10^{-4}$  or  $10^{-5}$ , and the mice died with typical symptoms of encephalitis on the 5th to 7th day.

*St. Louis virus.* Through the kindness of Dr. Webster of the Rockefeller Institute, specimens of mouse brain infected with St. Louis virus number 3 were sent to us. The virus was active in high dilution, and Swiss mice (derived from the stock of the Rockefeller Institute) as well as local stock laboratory mice were found to be susceptible.

*Case 1.* Neutralization tests were carried out with both the St. Louis and the Japanese viruses according to the technique described by Webster and his coworkers (4) with the serum of Case 1, obtained about  $1\frac{1}{2}$  years after recovery. The serum obtained from Case 3 in March 1936, 5 months after recovery, was tested at the same time. The serum obtained from a child who had had as far as we could tell, no contact with encephalitis, was used as a control. Serum from one patient thought to have post-measles encephalitis, and also from another child with obscure symptoms suggesting subacute encephalitis, were included in some of the tests.

The same technique was used throughout the tests. The brain of one or more mice which had died with typical symptoms following the intracerebral injection of either the Japanese (Hashimoto) virus or the St. Louis virus were emulsi-

fied in broth so as to make a suspension of approximately 10 per cent by weight. In some instances the brains of the mice were stored in 50 per cent glycerine for 1 to 4 days before use. Dilutions ranging from  $10^{-2}$  to  $10^{-5}$  were made in broth from the 10 per cent emulsion. Equal quantities of the sera to be tested and the virus dilutions were mixed and incubated at  $37^{\circ}$  C. for 2 hours. Four mice were then injected intracerebrally with 0.03 cc. of each virus-serum mixture. Usually 12 mice were used for each serum. No significant difference in susceptibility was noted between Swiss mice and stock laboratory mice. Both types of mice were used in these experiments. Only a few representative protocols are given.

TABLE I  
*Protection test: Japanese virus (Hashimoto).  
January 4, 1936*

Serum \ Dilution	$10^{-2}$	$10^{-3}$	$10^{-4}$	Result
Case 1 Bled January 4, 1936...	4, 14*	6	11	Protection (8 survived)
Case 2 Bled December 3, 1935	7, 7, 7, 7	7, 8, 8	6, 9	No protection (3 survived)
Questionable post-measles encephalitis.	6, 6, 6	6, 6, 7, 15	5, 15, 16	No protection (2 survived)
Non-contact serum...	6, 7, 7	6, 7, 7, 8	6, 6, 8	No protection (2 survived)

\* Duration of life of mouse in days; blanks indicate that mice remained well; mice were observed for a period of 2 weeks or longer.

TABLE II  
*Protection test: Japanese virus (Hashimoto).  
February 13, 1936*

Serum \ Dilution	$10^{-2}$	$10^{-3}$	$10^{-4}$	Result
Case 1 Bled January 4, 1936...	8* (Only 3 mice used for this dilution)	2	2	Protection (7 survived)
Non-contact serum...	6, 6, 6, 6	6, 6, 6, 7	6, 6, 7	No protection (1 survived)

\* See footnote to Table I.

The serum from Case 1 was found to protect against both the Japanese and the St. Louis virus. The sera obtained from Case 3 and the non-contact serum failed to protect. These experiments were repeated with the same results. The sera from the case of possible post-measles encephalitis and that from the case of atypical encephalitis also failed to protect.

TABLE III

*Protection test: St. Louis virus. March 19, 1936*

Serum	Dilution	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	Result
Case 1 Bled January 4, 1936...	18, 18*				Strong protection (10 survived)
Case 3 Bled March 17, 1936...	5, 6, 7, 7	3, 6, 6, 7	6, 7, 7		No protection (1 survived)
Non-contact serum	5, 5, 7, 8	6, 6, 7, 7	7, 7, 7, 15		No protection (None survived)
Atypical encephalitis...	4, 5, 5, 6	6, 6, 8	6, 6, 6, 7		No protection (1 survived)

\* See footnote to Table I.

TABLE IV

*Protection test: St. Louis virus. April 1, 1936*

Serum	Dilution	10 <sup>-2</sup>	10 <sup>-3</sup>	Result
Case 1 Bled January 4, 1936.....	4, 9*	1, 4		Protection (4 sur- vived)
Case 3 Bled March 17, 1936.....	7, 7, 7, 8	5, 6, 6, 6		No protection (None survived)

\* See footnote to Table I.

Case 2. Neutralization tests with the sera obtained from Case 2 were only made with the Japanese virus, since the supply of this patient's sera was exhausted before the St. Louis virus was available. Both the serum obtained during the acute stage and that obtained during convalescence failed to protect.

*Cross-protection tests.* The fact that the serum of Case 1 protected against both the Japanese (Hashimoto) and the St. Louis virus seemed to indicate that these two viruses might be closely related. It was thought of interest to see whether mice that had survived inoculation with one of these viruses were immune to intracerebral injection of the other virus. Five mice which had been inoculated with the Japanese virus on March 19th and had survived, were inoculated with St. Louis virus diluted 10<sup>-2</sup> on April 1st. All 5 mice remained well, whereas 4 control mice inoculated with the same dilution of virus died with typical symptoms 7 days after inoculation.

A few mice which had survived intracerebral injection with the St. Louis virus were reinoculated intracerebrally with the Japanese virus diluted 10<sup>-4</sup>. The mice which had been previously inoculated with the St. Louis virus survived, whereas the control animals died on the 6th to 7th day following injection.

## DISCUSSION

Case 1, as far as we could determine, contracted encephalitis in China. She had, to our knowledge, no contact with any individuals who had recently come from Japan. No other cases of encephalitis developed in the community where she was living. This patient's serum, obtained 1½ years after recovery, contained neutralizing antibodies both for the virus isolated in Japan in 1935 by Hashimoto and for the virus isolated by Webster in America during the St. Louis epidemic of 1933. Mice that survived inoculation with the Japanese virus, were immune to subsequent intracerebral injections with the St. Louis virus, and *vice versa*. Due to a shortage of mice, the number of animals in the cross protection tests is small, but the results are suggestive. These experiments seem to indicate that the Japanese virus isolated in 1935 and the St. Louis virus isolated in 1933 may be closely related.

The failure of the serum from Case 2 to protect against the Japanese virus is of interest because this patient obviously contracted encephalitis in Japan. It may be that a later bleeding will show protective antibodies.

The lack of protective antibodies in the serum obtained from Case 3 seems to exclude the virus of epidemic encephalitis, either of the St. Louis or the Japanese type, in this case. Attempts to demonstrate vaccine virus in the spinal fluid of this patient failed. Although vaccine encephalitis is rare following re-vaccination, it has been observed (8). The incubation period following vaccination was typical in this patient.

Both the cases of epidemic encephalitis described, seem to show clear-cut sequelae. In Case 1, personality changes and emotional instability have persisted for more than 1½ years. In Case 2, there was an unusually severe and protracted course. This patient retains a foot-drop and an impaired memory.

## SUMMARY

1. Two cases of epidemic encephalitis have been observed in North China which were followed by clear-cut sequelae.

2. The serum of one of these cases contained protective antibodies both against the St. Louis type of encephalitis virus and against a virus iso-



lated during the 1935 encephalitis epidemic in Japan by Hashimoto.

3. A small number of cross protection tests suggest that injections with the Japanese (Hashimoto) virus protect against the St. Louis type of virus, and *vice versa*.

4. A case of possible vaccine encephalitis in a Chinese child is described.

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# HEMOPHILIA. I. THE ABNORMAL COAGULATION OF THE BLOOD AND ITS RELATION TO THE BLOOD PLATELETS

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The one abnormality constantly found in hemophilia is an inability of the blood to coagulate in a normal manner. This defect is demonstrable usually by a prolonged clotting time, which in turn determines the various manifestations of the disease. Further understanding of this disease depends, therefore, upon knowledge of the cause of the prolonged clotting time. Most investigations of the blood clotting mechanism in hemophilia have been made with methods involving the recalcification of oxalated or citrated plasma, and have been interpreted in terms of the several theories of blood coagulation. In the present study this approach to the problem has been abandoned for two reasons: (1) the recalcification of plasma is an artificial, indirect procedure which may not reflect physiological changes; (2) the theories of blood coagulation, with their varied nomenclature, possibly have served to confound rather than to clarify understanding of this disease.

The present study is based upon the well recognized observation that transfusion with normal blood reduces the clotting time of whole blood in hemophilia. It was assumed, therefore, that normal blood contains a substance which either supplies a clotting factor lacking from hemophilic blood or which counteracts a mechanism inhibiting coagulation of the blood in hemophilia. The analysis of the nature of this substance or substances contained in normal blood has been the aim of these observations. Since such inquiry has been made by many workers, certain of the observations recorded here are repetitious of other studies in this field. However, these differed in methods, concepts, or interpretations from the present work. A critical evaluation of the views of other investigators will be reserved for later discussion.

## METHODS

Normal blood was obtained by venipuncture and was mixed gently with such an amount of 2.5 per cent sodium

citrate in distilled water that the final concentration was 0.25 per cent. Conditions for the observation of clotting times were kept rigidly uniform. Acid-cleaned test tubes 100 × 12 mm. were used. The test blood or blood constituent was pipetted to the bottom of the tube, which had been freshly rinsed with 0.85 per cent solution of sodium chloride. Hemophilic blood was then obtained by venipuncture, the needle of the syringe removed, and exactly 2 cc. added to the tube containing the test material—discarding, however, the last three or four cubic centimeters of blood in the syringe. Control clotting times were made by adding hemophilic blood to tubes containing normal saline in amounts similar to the test material. The adding of hemophilic blood in this manner provided adequate mixing. Shaking the tubes was avoided, since such agitation provoked irregular responses. The tubes were then immersed directly in a water bath at 37° C. After five minutes and at intervals thereafter, they were examined by gently tilting in order to see if the surface had jelled sufficiently to sustain the blood when the tube was inverted. The time after venipuncture at which this occurred was read as the clotting time. With such precautions a remarkably close check was obtained with duplicate observations, which invariably were made.

Except when otherwise stated, this procedure was followed in the experiments to be described, and henceforth will be referred to as "standard technique." It is essential, in making comparative readings on clotting time to have uniformity in the size of the tubes, in the amount of blood, and in the temperature. It is essential also that the syringe be clean and well rinsed with saline, and that the venipuncture be directly successful. Admixture with tissue juice, as in puncture by "second intention," may give falsely reduced clotting times. We believe that in such studies it is desirable to make observations on patients with decidedly protracted clotting time, so that any reduction in time may be considerable. A clotting time of one to two hours at 37° C. may be the equivalent of 5 to 10 hours at room temperature, and whereas duplicate tests done at room temperature may be widely variant, those done at 37° C. agree within a few minutes of one another. For the sake of brevity and simplicity, only a few characteristic observations out of many are reported in this paper.

## I. Studies with whole blood

*Effect on clotting time of hemophilic blood of adding citrated normal blood in vitro.* When varying amounts of citrated normal blood were added to 2 cc. of fresh hemophilic blood, it was found that as little as 0.05 cc. of normal blood, or a dilution of 1:40, was optimally effective in reducing the clotting time of the hemophilic blood (Table I). Therefore greater dilution was tried

TABLE I

*Observations on clotting time after adding citrated normal blood to 2 cc. of hemophilic blood in standard tubes (100 × 12 mm.)*

Citrated normal blood	Clotting times		
	Case I	Case II	Case III
cc.	minutes	minutes	minutes
0.0	28	90	29
0.05	7	7	9
0.1	6	7	9
0.2	7	6	9

by adding like amounts of normal blood to 4 cc. of hemophilic blood. For this purpose, larger tubes, 110 × 14 mm. were necessary in place of the usual standard tubes. Here, too, the smallest amount of normal blood added was optimally effective in reducing the clotting time of hemophilic blood, even though the ratio of volumes between normal and hemophilic blood was 1:200 (Table II).

TABLE II

*Observations on clotting time after adding citrated normal blood to 4 cc. of hemophilic blood in larger tubes (110 × 14 mm.)*

Citrated normal blood	Clotting times		
	Case I	Case II	Case III
cc.	minutes	minutes	minutes
0.0	170	60	90
0.02	20	23	27
0.05	15	23	16
0.1	15	30	22

*The effect on the clotting time of normal blood of adding citrated hemophilic blood in vitro.* In each of several standard tubes were pipetted graded amounts of citrated hemophilic blood. Thereupon 2 cc. of normal blood were added to each tube. However, the clotting time of the normal blood remained unchanged (Table III).

TABLE III

*Observations on clotting time after adding graded amounts of citrated hemophilic blood to 2 cc. of normal blood \**

	Clotting time
	minutes
2 cc. normal blood.....	11
2 cc. normal blood + 0.03 cc. hemophilic blood....	12
2 cc. normal blood + 0.05 cc. hemophilic blood....	11

\* Hemophilic blood had clotting time of 2 hours.

*Effect of adding citrated hemophilic blood to another hemophilic blood in vitro.* In each of several standard tubes were pipetted (a) graded amounts of citrated hemophilic blood whose clotting time was 35 minutes, and (b) in another series of tubes were pipetted similar amounts of citrated hemophilic blood with a clotting time of 105 minutes. To each of the tubes was then added 2 cc. of the control or test hemophilic blood whose clotting time was 25 minutes. No significant change in the latter's clotting time occurred (Table IV).

TABLE IV

*Observations on clotting time of hemophilic blood after adding graded amounts of another hemophilic blood \**

	Clotting time
	minutes
2 cc. control hemophilic blood.....	25
2 cc. control hemophilic blood + 0.03 cc. hemophilic blood A.....	25
2 cc. control hemophilic blood + 0.05 cc. hemophilic blood A.....	25
2 cc. control hemophilic blood + 0.03 cc. hemophilic blood B.....	30
2 cc. control hemophilic blood + 0.05 cc. hemophilic blood B.....	30

\* Hemophilic blood A had clotting time of 35 minutes. Hemophilic blood B had clotting time of 105 minutes.

*Effect of transfusion with normal citrated blood on the clotting time of hemophilic blood.* Normal blood, when transfused into patients with hemophilia, abruptly reduced the clotting time. This occurred with amounts as small as 30 cc. and the effect could be detected five to ten minutes after transfusion. As in the test tube, whether this normal blood represented to the patient's calculated blood volume a ratio of 1:8 or 1:53, an approximately equivalent reduction in clotting time was observed. The smaller amounts, however, provided usually a more transient effect than the larger amounts. The clotting time gradually

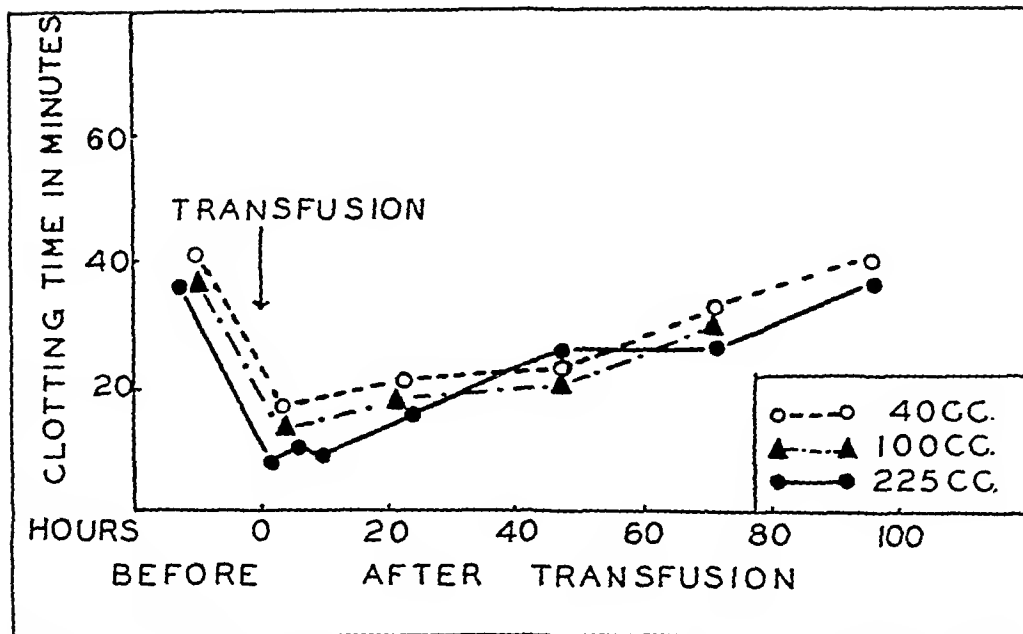


FIGURE 1. OBSERVATIONS ON THE CLOTTING TIME OF HEMOPHILIC BLOOD AFTER TRANSFUSION WITH VARYING AMOUNTS OF CITRATED NORMAL BLOOD (CASE I).

increased, in most instances reaching and surpassing the initial level in three to four days. Normal citrated blood kept three days at ice-box temperature (5 to 10° C.) was equally effective (Figures 1 and 2).

There is a substance in normal blood which in small quantity effectively reduces the clotting time of hemophilic blood, both *in vitro* and *in vivo*. This substance is stable at ice-box temperature. Since the addition of hemophilic blood to normal blood does not prolong the clotting time of normal blood, it appears that the defective coagulation in hemophilia is not due to an inhibitory agent. Since the addition of one hemophilic blood to another did not alter the clotting of the control or test blood, it may be assumed that the previous changes observed on the addition of normal blood were specific and were not due to the mechanical admixture of two blood samples.

## II. Studies with blood components

*Comparison of blood, plasma, and serum in vitro.* Comparison was made of equivalent amounts of citrated normal blood, plasma, and serum in their clot-accelerating action on hemophilic blood. Normal blood was obtained by venipuncture. One portion of this was citrated by

standard technique. A second portion was citrated, then centrifuged immediately at 1,500 r.p.m. for 20 minutes, and the plasma removed. A third uncitrated portion was allowed to clot at room temperature, then centrifuged immediately at 1,500 r.p.m. for 20 minutes, and the serum removed. Manipulation was done by sterile technique. Tests were made at varying intervals of time of the relative potency of these portions in reducing the clotting time of hemophilic blood. When not in use, the blood, plasma, and serum were kept at ice-box temperature. Tests were made by pipetting 0.1 cc. of citrated normal blood, 0.05 cc. of plasma or of serum to the bottom of standard tubes. To each of these were added 2 cc. of fresh hemophilic blood. It is evident from the data given in Table V that whole blood was the most effective in reducing the clotting time of hemophilic blood, that plasma was slightly less so, and that serum, although somewhat active when fresh, lost its potency after several days.

*Effect of transfusion with normal serum on the clotting time of hemophilic blood.* The intravenous injection of serum was done twice. In one case the normal blood was allowed to clot at room temperature, and after removal and Berkefeld filtration, the serum was kept at ice-box tem-

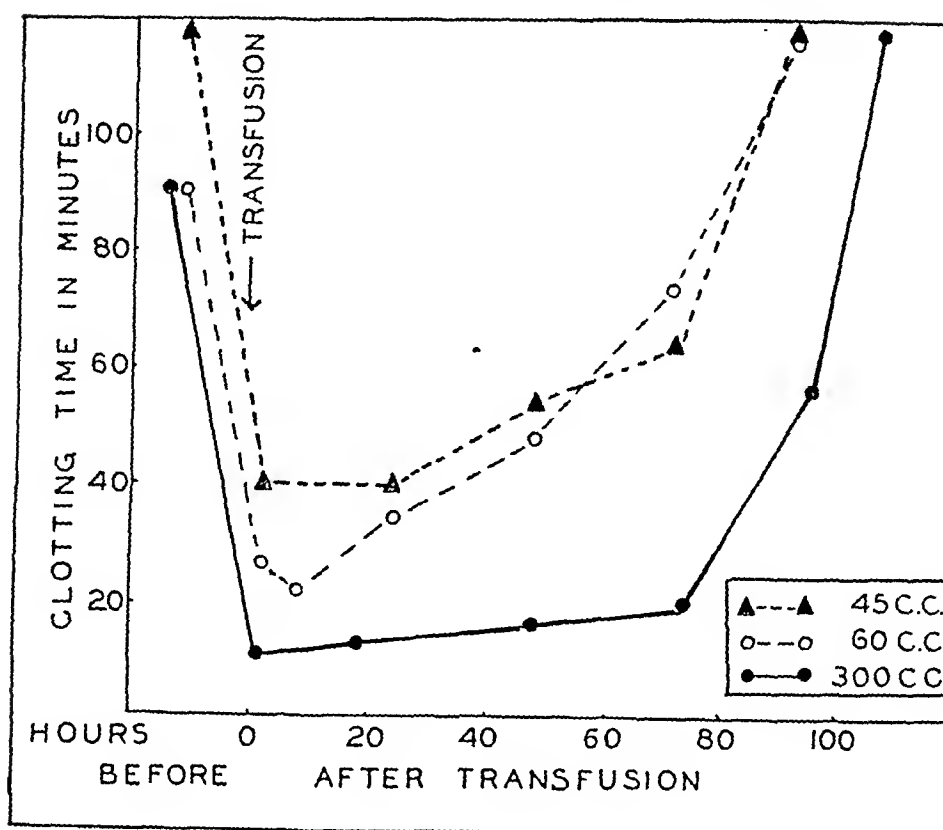


FIGURE 2. OBSERVATIONS ON THE CLOTTING TIME OF HEMOPHILIC BLOOD AFTER TRANSFUSION WITH VARYING AMOUNTS OF CITRATED NORMAL BLOOD (CASE II).

TABLE V

Observations on clotting time of hemophilic blood after adding citrated normal blood, plasma, and serum in equivalent amounts at varying intervals of time

Case number	Clotting time				Age of test material
	Control hemophilic blood	0.1 cc. blood	0.05 cc. plasma	0.05 cc. serum	
I	minutes	minutes	minutes	minutes	
	105	14	26	32	2 hours
	105	12	14	35	4 days
	100	15	26	38	8 days
II	60	25	28	80	11 days
	150	14	26	32	2 hours
	150	20	25	55	2 days
	130	11	12	60	4 days
	130+	15	27	130+	8 days

perature for four days. Intravenous injection of this serum to a patient with hemophilia was followed by no reduction of clotting time. In a second case a similar procedure was performed with normal serum which had formed at ice-box temperature and had remained in contact with the clot for two days. After Berkefeld filtration, this serum was injected intravenously on the third

day. No change in clotting time of hemophilic blood occurred (Table VI). *In vitro* studies made with these sera according to standard technique were likewise negative.

TABLE VI

Observations on clotting time of hemophilic blood after transfusion with aged normal serum

	Clotting time	
	A*	B*
	minutes	minutes
Before injection.....	30	180
2 hours after injection.....	33	180
4 hours after injection.....	35	120
8 hours after injection.....		120

\* (A) 100 cc. serum 3 days old. (B) 35 cc. serum 4 days old.

These findings are in contrast to the action of fresh serum injected intravenously, as noted also by Addis (6a) and likewise in contrast to our *in vitro* studies with fresh serum, which had the ability to hasten clotting of hemophilic blood.

Thus, whereas this clot-accelerating factor was stable in both blood and plasma, the serum effect was peculiarly labile. Attention, therefore, was

directed to the plasma and its constituents, since preliminary studies indicated that its activity more closely paralleled that of whole blood.

*In vitro studies with plasma subjected to filtration and protracted centrifugation.* Fresh normal blood, obtained by venipuncture, was placed directly in paraffin-lined centrifuge tubes containing sodium citrate. Plasma was removed by standard technique, with the exception that all materials used in the manipulation were paraffin-coated. A portion of the plasma was kept whole, and a second portion was filtered through six thicknesses of number 42 filter paper. Of these portions one-half was centrifuged at 3,000 r.p.m. for 45 minutes, and the top layer used for testing. In contrast to whole plasma, centrifuged whole and filtered plasmas contained by microscopic observation very few platelets. The relative effectiveness of these plasmas was then tested by standard technique against hemophilic blood.

Filtration and centrifugation resulted in little, if any, loss of potency (Table VII). The addi-

TABLE VII

*Observations on clotting time of hemophilic blood after adding normal platelet-poor plasma, as obtained by filtration or centrifugation*

	Clotting time	
	Case I	Case II
	minutes	minutes
2 cc. control hemophilic blood.....	55	120
2 cc. control + 0.02 cc. whole normal plasma.....	15	25
2 cc. control + 0.02 cc. filtered normal plasma.....	20	30
2 cc. control + 0.02 cc. top layer centrifuged whole normal plasma.....	20	30
2 cc. control + 0.02 cc. top layer centrifuged filtered normal plasma.....	20	35

tion of either plasma caused prompt reduction in the clotting time of hemophilic blood. Similar observations were made with oxalated plasma, and the results obtained were essentially the same. Whereas the dilution of these plasmas in hemophilic blood (.02 in 2 cc.) was about 1:100, similar observations were made with equal volumes of plasma which had been diluted with saline in order to make a relative dilution of 1:200 and of 1:300. There was a tendency towards slight reduction of clot-promoting power of these plasmas with higher dilutions.

*In vitro studies with plasma of purpura blood.*

Fresh citrated plasma was obtained from normal subjects and was compared to that obtained from patients with thrombopenic purpura. These patients characteristically revealed prolonged bleeding time, and their blood showed extreme thrombopenia and non-retractile clot. Their plasma was used because, without being subjected to manipulation, it contained very few platelets. One case was due to arsphenamine therapy; one was associated with a late phase of aplastic anemia; two were instances of idiopathic thrombopenic purpura. Tests were made by standard technique of the relative effectiveness of such platelet-poor plasmas as compared to that of normal subjects. No demonstrable differences were found in the behavior of the plasmas from the various types of purpura, which were as effective as normal plasma in reducing the clotting time of hemophilic blood. An example of the potency of the respective plasmas is illustrated by Table VIII.

TABLE VIII

*Observations on clotting time of hemophilic blood after adding citrated plasma of normal subject and of patient with thrombopenic purpura*

Plasma	Clotting time			
	Normal plasma		Purpura plasma	
	Case I	Case II	Case I	Case II
cc.	minutes	minutes	minutes	minutes
0.0	30	120	30	120
0.02	6	25	8	28
0.05	5	15	7	18
0.1	5	18	6	11

*In vitro studies with normal plasma passed through the Berkefeld filter.* Fresh citrated normal plasma was obtained by standard technique. One portion was kept as such; another was passed through a Berkefeld filter.<sup>1</sup> The latter was entirely free from cellular constituents. Standard tests were made with like amounts of the plasmas so prepared, after being stored at 8° C. for four days and thirty days. Both retained activity. It was observed that the clot-accelerating action of whole normal plasma was not affected significantly by filtration (Table IX).

<sup>1</sup> Hereafter in this paper the term "filtered" will denote passage through the Berkefeld filter (Grade V) unless otherwise specified.

TABLE IX

*Observations on clotting time of hemophilic blood after adding normal citrated plasma, whole or filtered*

Plasma	Clotting time			
	Whole normal plasma, 4 days old		Filtered normal plasma, 4 days old	
	Case I	Case II	Case I	Case II
	minutes	minutes	minutes	minutes
cc.				
0	32	90	32	90
0.02	15	36	20	40
0.05	13	36	17	37
0.1	11	30	11	30
0.3	11	30	11	30
	Whole normal plasma, 30 days old		Filtered normal plasma, 30 days old	
0		120		120
0.1		30		35

*In vitro* comparison of normal and hemophilic plasmas. Whole and filtered plasma of citrated normal and hemophilic blood respectively were compared by standard technique in their clot-promoting power on another hemophilic blood. As observed previously, normal plasma, whether it was whole or filtered, reduced the clotting time of hemophilic blood, whereas this power was either absent from or greatly diminished in hemophilic plasma (Table X).

TABLE X

*Observations on clotting time of hemophilic blood after adding normal and hemophilic plasma of another patient*

	Clotting time	
	Case I	Case II
	minutes	minutes
2 cc. control hemophilic blood.....	120	75
2 cc. control + 0.03 whole normal plasma...	28	25
2 cc. control + 0.03 whole hemophilic plasma...	120	75
2 cc. control + 0.03 filtered normal plasma..	28	25
2 cc. control + 0.03 filtered hemophilic plasma.....	120	75

*The effect of transfusion with normal filtered plasma on the clotting time of hemophilic blood.* Single intravenous injections of citrated filtered normal plasma reduced the clotting time of hemophilic blood for several hours. The plasma used was kept from three days to two months at ice-box temperature. On one occasion this plasma was used to check hemorrhage. Transfusion with 150 cc. of such plasma, two months old,

apparently stopped a protracted nosebleed in 10 minutes and reduced the clotting time from 2 hours to thirty minutes, 4 hours after the injection. A similar response was obtained by the intravenous injection of citrated plasma from a patient with thrombopenic purpura.

By giving daily intravenous injections of 75 to 100 cc. of filtered normal plasma for three days to a hemophilic patient, the clotting time was kept materially reduced during this time. The response with plasma was similar in pattern to but less durable than that of whole blood transfusion in these patients (Figures 3 and 4).

*In normal plasma rendered free from platelets, whether by filtration or centrifugation, there is a substance which is effective in reducing the clotting time of hemophilic blood. This substance also resides in the plasma of thrombopenic purpura. It is stable at ice-box temperature and is potent in high dilution in vitro or in vivo. It is either unavailable or greatly diminished in hemophilic plasma.*

### III. Study of the blood platelets in relation to the clotting of normal and hemophilic blood

Since plasma, free from platelets, contained material effective in reducing the clotting time of hemophilic blood, several questions presented themselves: (1) was such plasma (filtered, for example) also free from platelet products, (2) did hemophilic platelets behave like normal platelets in their own plasma and in normal plasma; (3) did the addition of platelets or platelet material free from plasma influence the clotting time of hemophilic blood? Such inquiry was pertinent because some current theories hold that the hemophilic platelets, due to an abnormal stability, break up slowly and consequently liberate slowly a substance effective in clotting. The method for obtaining platelet material was as follows: 50 cc. of plasma was derived from citrated normal blood by standard technique. This was centrifuged at 1,500 r.p.m. for 20 minutes, the cloudy plasma removed, and then divided into two equal portions and recentrifuged at 2,000 r.p.m. for 2 hours. The white sediment at the bottom, which was composed of platelets, was washed twice with 0.85 per cent sodium chloride solution. One portion was resuspended

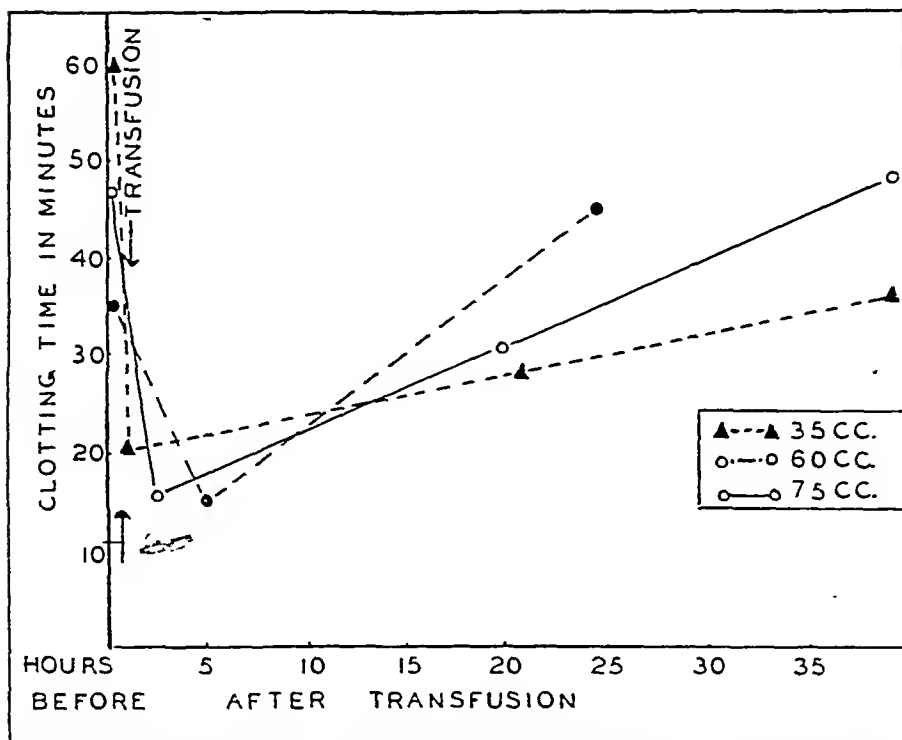


FIGURE 3. OBSERVATIONS ON THE CLOTTING TIME OF HEMOPHILIC BLOOD AFTER SINGLE TRANSFUSION WITH FILTERED NORMAL PLASMA (CASE III).

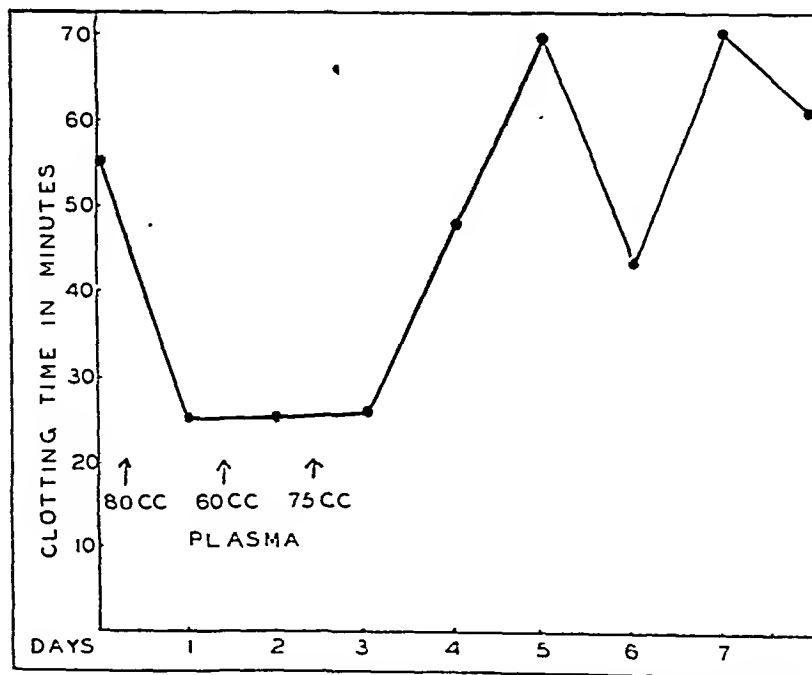


FIGURE 4. OBSERVATIONS ON THE CLOTTING TIME OF HEMOPHILIC BLOOD AFTER MULTIPLE TRANSFUSIONS WITH FILTERED NORMAL PLASMA (CASE III).



TABLE XI

*Observations on clotting time of normal and hemophilic filtered plasma after the addition of normal and hemophilic platelet suspensions \**

A. HEMOPHILIC PLASMA			
		Clotting time <i>minutes</i>	
Whole plasma.....			57
Filtered plasma.....			150
		Hemophilic platelets	Normal platelets
Filtered plasma + saline suspension of platelets.....		60	62
Filtered plasma + saline filtrate of platelets.....		150	150
Filtered plasma + water suspension of platelets.....		70	69
Filtered plasma + water filtrate of platelets.....		150	150
B. NORMAL PLASMA			
		Clotting time <i>minutes</i>	
Whole plasma.....			6
Filtered plasma.....			30
		Hemophilic platelets	Normal platelets
Filtered plasma + saline suspension of platelets.....		6	9
Filtered plasma + saline filtrate of platelets.....		28	30
Filtered plasma + water suspension of platelets.....		7	9
Filtered plasma + water filtrate of platelets.....		16	19

\* To 0.2 cc. of each of the above plasmas were added successively 0.1 cc. of platelet suspension of physiological concentration and then 0.9 cc. of a "calcium solution." Each tube was shaken briefly to effect mixture and then immersed in a water bath at 37° C.

in saline of the same volume as the plasma from which it was derived. The second portion was similarly resuspended in distilled water. After vigorous shaking to afford an even suspension of platelets each of these portions, in turn, was divided—part kept whole and part passed through the Berkefeld filter.

An exactly similar procedure was carried out with an equal volume of hemophilic plasma. Thus there resulted stoichiometric suspensions of normal and hemophilic platelets in water and in physiological saline, and of their respective filtrates.

*Comparison of hemophilic and normal platelets in both hemophilic and normal plasmas.* Comparison between the effects of plasma and of platelets from both hemophilic and normal blood was made by use of the method of recalcification of citrated plasma. This involved the addition to each tube successively of (1) 0.2 cc. of the test plasma; (2) 0.1 cc. of platelet suspension; and (3) 0.9 cc. of calcium solution.<sup>2</sup> Then the tubes were shaken briefly and placed in the water bath at 37° C.

<sup>2</sup> Calcium solution was made up in lots composed of 80 cc. of 0.85 per cent NaCl + 25 cc. of 0.25 per cent CaCl<sub>2</sub>.

Observations are recorded in Table XI. The clotting of whole hemophilic plasma on recalcification required 57 minutes whereas the same plasma when filtered and recalcified required 150 minutes. Presumably this was due to loss of platelets. The addition of hemophilic or normal platelet suspensions to the filtered hemophilic plasma brought the clotting time back approximately to that of whole hemophilic plasma. A similar prolongation of clotting time occurred after Berkefeld filtration of normal plasma. Here, too, the addition of hemophilic or of normal platelet suspensions brought the clotting time back to that of whole normal plasma. Under the above conditions, hemophilic platelets provided the same accelerating effect as did normal platelets when added either to their own or to normal filtered plasma.

The platelet suspensions were then subjected to filtration. Their filtrates were added in a similar manner to filtered plasma from normal and from hemophilic blood. No accelerating effect was observed. Hence it was evident that filtration of platelets, however injured they were by water lysis and manipulation, allowed the transfer of virtually no clot-accelerating material into the filtrate. Therefore, it may be a general

plasma, subjected to filtration, was also practically free from platelets and platelet products.

*Comparison of normal and hemophilic plasma method of recalcification.* With the use of technique described above, normal and hemophilic plasmas, whole and filtered, were tested for clotting time on recalcification. Again it was found that Berkefeld filtration with removal of platelets, caused prolongation of both normal and hemophilic plasma clotting time. However, adding 0.1 cc. of filtered normal plasma (clotting time 40 minutes) to 0.1 cc. of whole hemophilic plasma (clotting time 24 minutes) the resultant clotting time was but 8 minutes—that is, essentially normal. This demonstrated that platelet-free normal plasma, when added to whole

hemophilic plasma, supplied a material effective in producing normal clotting time on recalcification (Table XII).

In order to determine whether normal plasma acted directly on hemophilic plasma, or acted indirectly by activating the platelets, the following experiment was done: graded small amounts of filtered normal plasma were added to filtered hemophilic plasma, and the clotting times noted after recalcification. By this procedure the clotting time of filtered hemophilic plasma was reduced to that of filtered normal plasma (Table XIII). These results indicate that the effectiveness of normal plasma in reducing the clotting time of hemophilic blood does not necessarily depend upon activation of platelets.

*Addition of platelet suspensions to hemophilic blood in vitro.* Observations on the recalcification of citrated plasma suggested that platelets were not related to the defective coagulation in hemophilia. Such studies, however, involved an unphysiological procedure. That is to say, the recalcification of citrated plasma did not reflect necessarily the changes occurring in natural clotting. Therefore, platelet suspensions of the concentration in normal plasma, as prepared in the preceding study, were added directly to whole hemophilic blood by standard technique, and the clotting times noted. Although the platelet suspension was of physiological concentration, the number of normal platelets relative to the total volume of hemophilic blood was considerably less than physiological concentration. This amount was used simply for comparison with a like

TABLE XII

*Observations on clotting time of normal and hemophilic plasmas upon adding 0.9 cc. of "calcium solution"*

	Clotting time
<b>A</b>	
cc. whole normal plasma	6
cc. whole hemophilic plasma	24
cc. filtered normal plasma	40
cc. filtered hemophilic plasma	210
<b>B</b>	
cc. whole hemophilic plasma	8
+ cc. filtered normal plasma	
cc. whole hemophilic plasma	20
+ cc. normal saline	
cc. filtered normal plasma	43
+ cc. normal saline	

TABLE XIII

*Observations on clotting time of filtered hemophilic plasma after adding filtered normal plasma \**

	Clotting time
	minutes
cc. filtered hemophilic plasma	270
cc. filtered hemophilic plasma + 0.01 cc. filtered normal plasma + 0.09 cc. saline	51
cc. filtered hemophilic plasma + 0.02 cc. filtered normal plasma + 0.08 cc. saline	53
cc. filtered hemophilic plasma + 0.04 cc. filtered normal plasma + 0.06 cc. saline	44
cc. filtered hemophilic plasma + 0.06 cc. filtered normal plasma + 0.04 cc. saline	39
cc. filtered hemophilic plasma + 0.08 cc. filtered normal plasma + 0.02 cc. saline	32
cc. filtered hemophilic plasma + 0.1 cc. filtered normal plasma	30

## CONTROLS

cc. filtered normal plasma + 0.1 cc. saline	36
cc. filtered normal plasma + 0.16 cc. saline	No clotting
cc. whole normal plasma + 0.1 cc. saline	8

\* To each tube was added 1 cc. of "calcium solution." The tubes were shaken briefly and then immersed in a water bath at 37° C.

TABLE XIV

*Observations on clotting time of hemophilic blood after adding suspensions of normal and hemophilic platelets*

	Clotting time	
	Case I	Case II
	minutes	minutes
2 cc. control hemophilic blood . . . . .	120	150
2 cc. control + 0.03 cc. saline suspension normal platelets . . . . .	120	150
2 cc. control + 0.03 cc. saline suspension hemophilic platelets . . . . .	120	150
2 cc. control + 0.03 cc. water suspension normal platelets . . . . .	120	150
2 cc. control + 0.03 cc. water suspension hemophilic platelets . . . . .	120	150
2 cc. control + 0.03 cc. saline filtrate normal platelets . . . . .	120	150
2 cc. control + 0.03 cc. saline filtrate hemophilic platelets . . . . .	120	150
2 cc. control + 0.03 cc. water filtrate normal platelets . . . . .	120	150
2 cc. control + 0.03 cc. water filtrate hemophilic platelets . . . . .	120	150

amount of platelet-free plasma, which had been shown to be optimally effective in reducing the clotting time of hemophilic blood. The addition of normal platelets *in this concentration* produced no change in the clotting time of the hemophilic blood (Table XIV).

*In the clotting of either hemophilic or of normal plasma, hemophilic platelets behave similarly to normal platelets. Filtration prolongs the clotting time of both normal and of hemophilic plasmas on recalcification. This is due presumably to the removal of platelets. However, the addition of filtered normal plasma to whole hemophilic plasma causes a sharp reduction of clotting time on recalcification. This indicates that the clot-promoting substance provided by normal blood resides in the platelet-free plasma. Moreover, the addition of a suspension of normal platelets of the concentration present in normal plasma to whole hemophilic blood causes no change in the latter's clotting time.*

#### DISCUSSION

It is not the purpose of this paper to review the mass of contradictory findings reported in the medical literature regarding the abnormality of blood coagulation in hemophilia. Convincing objections to the older theories of Wright (1), Sahli (2), Emile-Weil (3), Nolf and Herry (4) and Morawitz and Lossen (5) were presented in the excellent paper by Addis (6). A copious bibliography was also included in more recent articles by Minot and Lee (7), Hurwitz and Lucas (8) and Christie, Davies and Stewart (9). The latter authors, whose observations were interpreted in Howell's terminology, reported a

long "prothrombin-time." This term, however, signified simply a long clotting time of hemophilic oxalated plasma on recalcification. In other words, it showed that plasma in hemophilia manifested the same clotting abnormality as that of the whole blood. Klinger (20) made similar observations. By exclusion of other factors it was assumed by these authors to represent a defect in the substance "prothrombin."

In our opinion only the studies of Addis (6) truly pointed with substantial reason to a defect in the plasma which was interpreted as a qualitative change in "prothrombin," a material closely allied chemically to fibrinogen, but whose exact chemical nature is obscure. Addis recorded a number of important observations: that hemophilic plasma added to normal plasma did not lengthen the latter's clotting time, and hence contained no inhibitory substance; that unlike plasma, the clot-accelerating action of serum was labile; that thromboplastic substances in strong concentration reduced the clotting time of both normal and hemophilic blood, whereas in weaker concentration they were capable of reducing only the clotting time of normal blood effectively. In the present study a similar inquiry was directed at the clotting abnormality of hemophilia by use of a more direct technique involving *in vivo* as well as *in vitro* studies, and it was ascertained that this defect in the plasma was independent of the formed elements of the blood.

The relationship of platelets to blood clotting has been of interest for many years. All observers agree that in shed blood, platelets play an important rôle in the inception of clotting. The removal of platelets, whether by centrifugation

(10, 11) or by Berkefeld filtration (12, 13) and the consequent delay in clotting illustrates the point. Whether or not they are a source of "prothrombin" as well as "thromboplastin" is not of immediate concern.

In 1916 Minot and Lee (7) found that the addition of concentrated suspensions of normal platelets, unlike hemophilic platelets, caused a sharp reduction in the clotting time of hemophilic plasma on recalcification, whereas both normal and hemophilic platelet suspensions were essentially equal in their ability to hasten the clotting time of normal plasma. Fonio (14) and Wöhlisch (15) made similar observations. This was interpreted to mean that hemophilic platelets were only slowly available for purposes of enhancing coagulation. That these platelets behaved differently in normal plasma and in hemophilic plasma we interpret as indicating a difference of plasma rather than of platelets. It is possible that the normal platelets had not been adequately rinsed free of plasma. This could explain their greater effectiveness when added to hemophilic plasma. Studies by Feissly and Fried (19) clearly support this explanation. They demonstrated that although normal platelets appeared to be more effective than hemophilic platelets in hastening the clotting of hemophilic plasma, this difference did not exist after heating both platelet suspensions to 60° C. The thromboplastic activity of platelets is relatively stable at this temperature, whereas the effective substance in platelet-free plasma, as will be described in a later communication, is thermolabile.

In 1926 Howell and Cekada (16) concluded that the earlier hypothesis by Howell (16a) of a reduction in the amount of prothrombin was wrong, and that their later studies indicated that "the prothrombin in hemophilic blood does not differ from that of normal blood either in its concentration or its properties." These authors noted that platelets of normal blood, after being shed, disintegrated and fused more rapidly than did platelets of hemophilic blood. From this it was inferred that hemophilic platelets are abnormally stable. In our opinion such an observation merely confirms the fact that clotting is slow, but it fails to reveal or to imply an abnormal stability of platelets.

Christie et al. (9) in 1927 noted that injury by lysis or by switching of hemophilic blood hastened its clotting. Birch (17) in 1932 and others have made similar observations, from which they concluded that the platelets were abnormally tough in hemophilia. Such an assumption does not appear justifiable. The mechanism of clotting is very complex, unstable, and easily disturbed by change in the physical environment. In the course of such manipulation surely many factors in the blood or plasma are altered, which conceivably could hasten clot production, and it is unsound to interpret the effects from trauma as due to a selective and simple lysis of platelets. Govaerts and Gratia (21) suggested in their preliminary study on one patient, that a plasma factor worked by activating the platelet, and that this factor was deficient in hemophilia. From the data presented in Table XIII this postulation does not seem tenable.

It is not implied that platelets are unimportant in coagulation, but it is inferred from our study that hemophilic platelets function as well as do normal platelets. One source of the confusion in the literature is the fact that most studies have been made with highly concentrated suspensions or emulsions of platelets. It is well known that concentrated suspensions of platelets, of thromboplastin, or cephalin hasten clotting of normal and hemophilic blood. Such an effect is not specific. However, when they were compared stoichiometrically—or in the concentration in which platelets normally exist in plasma—then, hemophilic platelets were shown to behave as do normal platelets, both in their own and in normal plasma. This finding has also been suggested by studies of Addis (6), Eagles (18) and Feissly and Fried (19).

More significant was our observation that the addition of such suspensions of platelets to hemophilic blood did not shorten its clotting time, whereas the addition of a minute quantity of platelet-free, normal plasma abruptly reduced the clotting time, indicating that the platelet was not involved in the clotting abnormality of hemophilia, and that the defect resided in the plasma. *In vivo* studies with filtered normal plasma supported this contention.

Further studies are being made towards the

identification of this effective substance contained in normal plasma.

### CONCLUSIONS

There is a substance present in normal plasma and markedly deficient or unavailable in hemophilic plasma which in small amounts effectively shortens the clotting time of hemophilic blood. This plasma substance is stable at ice-box temperature. It is independent of the formed elements of the blood.

For their ready cooperation, the authors are deeply indebted to four hemophilic patients: Russell White, Edward Woogmaster, James Smith, and Victor Marotta.

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# EXPERIMENTAL HYPERTENSION—OBSERVATIONS ON SUSTAINED ELEVATION OF SYSTOLIC AND DIASTOLIC BLOOD PRESSURE IN DOGS

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The study of hypertension by animal experimentation has been hampered by the absence of satisfactory means of producing sustained elevations of blood pressure, and by the lack of accurate methods of recording systolic and diastolic blood pressures in the intact unanesthetized animal.

Recently, however, Goldblatt and his coworkers (1) have described an excellent method for the production of sustained increase in systolic blood pressure in dogs. Hypertension was produced by these authors by reducing the blood flow to the kidneys through the application of special silver clamps to both renal arteries; increases in systolic blood pressures, as recorded by the Van Leersum carotid loop method (2), were observed for as long as fifteen months after operation. Further, one of us, in a recent study (3), by using a sphygmographic blood pressure assembly originally described in principle by Erlanger (4) and later modified by Kolls (5), was able to record successfully systolic and diastolic hypertension in the unanesthetized dog. Although increases in systolic blood pressure have been reported, very few studies of diastolic blood pressures by acceptable methods are available in experimental hypertension, and even these are not of sufficiently long duration.

This communication presents studies on the effects of renal ischemia obtained by application of Goldblatt clamps, on the effects of ligation of the renal artery, and of partial nephrectomy on both the systolic and diastolic blood pressure in the unanesthetized dog. A further modification of the method utilized by Kolls and Cash (6) for measuring blood pressure in the unanesthetized dog is described.

Eight dogs, after suitable control periods, have been subjected to the following procedures: three animals have had Goldblatt clamps applied to the

renal arteries, two have had partial nephrectomy, and three, ligation of the renal artery. Numerous observations on systolic and diastolic pressure have been made at appropriate intervals over periods of five to twenty-eight months. Seven additional animals have been subjected to renal ischemia of short duration produced by the application of Goldblatt clamps. In the prolonged experiments occasional blood nonprotein nitrogen, twenty-four hour water intake, urinary output, and determinations of the specific gravity of the urine have served to indicate major changes in renal function.

## METHODS

Large and medium size dogs of mixed breeds have been used. They have been fed on prepared dog food (protein content approximately thirty per cent), supplemented by appropriate addition of cod liver oil from time to time. Water has not been limited to any specific amount except when the animals have been placed in metabolism cages for twenty-four hour studies of the urine at which time food was withheld and water measured and limited. Animals have been housed in individual cages and kept for weeks under laboratory conditions prior to the experimental period. The temperature of the room, in which blood pressure measurements were taken, varied between 70° and 78° F., except for somewhat higher temperatures obtaining at times during the summer months. The measurements of blood pressure were made on unanesthetized dogs by two observers.

### *Method of determination of blood pressure*

The method which was utilized for measuring blood pressure employs the usual sphygmomanometer assembly with the special sphygmograph designed by Kolls (5). This apparatus has been tested repeatedly in the unanesthetized dog by Kolls and Cash (6) who proved the accuracy of the method by simultaneous direct measurement of systolic and diastolic pressures with the maximum-minimum valve, in dogs which received both morphia and ether anesthesia.

An additional modification to the apparatus has been made by us which has proved practical and satisfactory. Owing to the difficulty of obtaining and keeping fresh

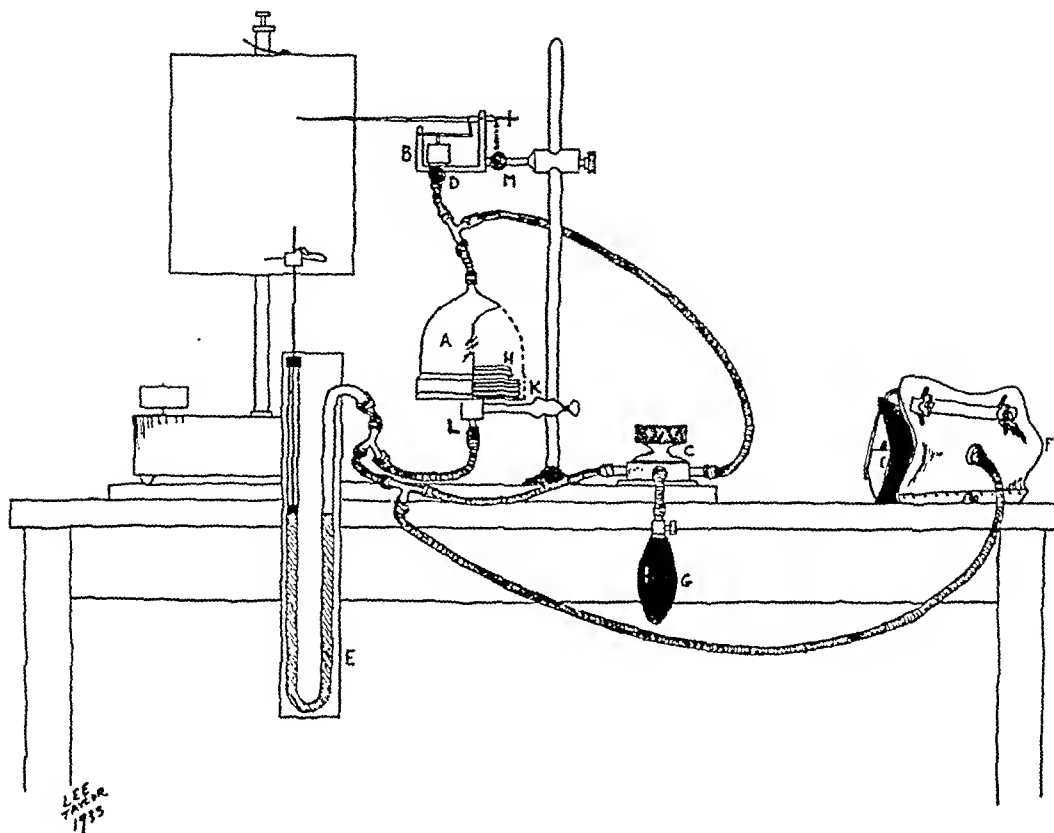


FIG. 1. ARRANGEMENT OF SPHYGMOMANOMETER ASSEMBLY.

*A*—Pressure bell; *B*—Kolls' sphygmograph; *C*—Three way valve; *D*—Escape valve of sphygmograph for adjustment of lever excursion; *E*—Mercury manometer; *F*—Metal and leather cuff containing rubber cuff for dog's leg; *G*—Bulb for inflation of cuff; *H*—Flange; *K*—Flanged base over which heavy rubber membrane is stretched; *L*—Outlet below rubber membrane connected with manometer cuff and three way valve; *M*—Screw adjusting lever against drum.

the original type of Erlanger bulb and to difficulties in recording low diastolic pressures with the more recent Erlanger and Meek sphygmoscope (7) we have substituted a pressure bell (*A*), Figure 1, measuring 8 cm. in diameter across the base over the flange (*H*) over which an unusually thick piece of automobile inner tubing (4.5 mm. thick) is tied with fine, strong cord. The bell is screwed on to the base (*K*), which is slightly raised above a lower outlet (*L*). This latter feature allows a delicate response to pulsations at extremely low diastolic levels, while at higher systolic levels the thick rubber diaphragm prevents overdistention into the bell toward the upper outlet. The measurements and shape of cuff (*F*), suitable for practically any dog, are essentially the same as those already described by Kolls.

The hair on the dog's leg should be kept closely clipped, the cuff accurately fitted as high as possible and maintained in this same position during the entire time of the tracing. As the pressure in the cuff is pumped up gradually, the dog may strain and tighten the leg muscles. Generally relaxation follows promptly and pressure may be recorded. Occasionally a very nervous animal may fail to respond to training.

Though this apparatus has proved highly satisfactory when properly used, we should like to point out that

experience with its numerous idiosyncrasies is necessary to obtain consistently accurate results. Dust, variations in temperature, and necessarily frequent handling may result in slight binding of the delicate joints or jewel bearings of the compound lever. Light weight, highly glazed paper, when smoked, frequently develops small, rough areas which interfere with the action of the lever. Therefore, a heavy variety of unglazed paper, lightly smoked is to be recommended. The lever should barely touch the drum, otherwise the small pulsations of systolic pressure will not be shown by a sudden increase in amplitude or the reduction in amplitude at the diastolic level will not be clearly reflected. Very accurate adjustment of the pressure of the lever against the drum is readily accomplished by the screw (*M*). We have found it best to regulate this pressure so that the lever writes in slightly broken lines (Figure 2). We have also found that the bakelite cap (*B*) (Figure 1) sometimes contracts or expands sufficiently to cause binding upon the underlying gold plated cylinder. This can be readily corrected by polishing with emery powder, or even slight reaming out with a small, sharp scotch. The final point at which serious difficulty may arise is in the three-way valve (*C*) which allows atmospheric pressure to be maintained in the bell above the rubber membrane both during inflation

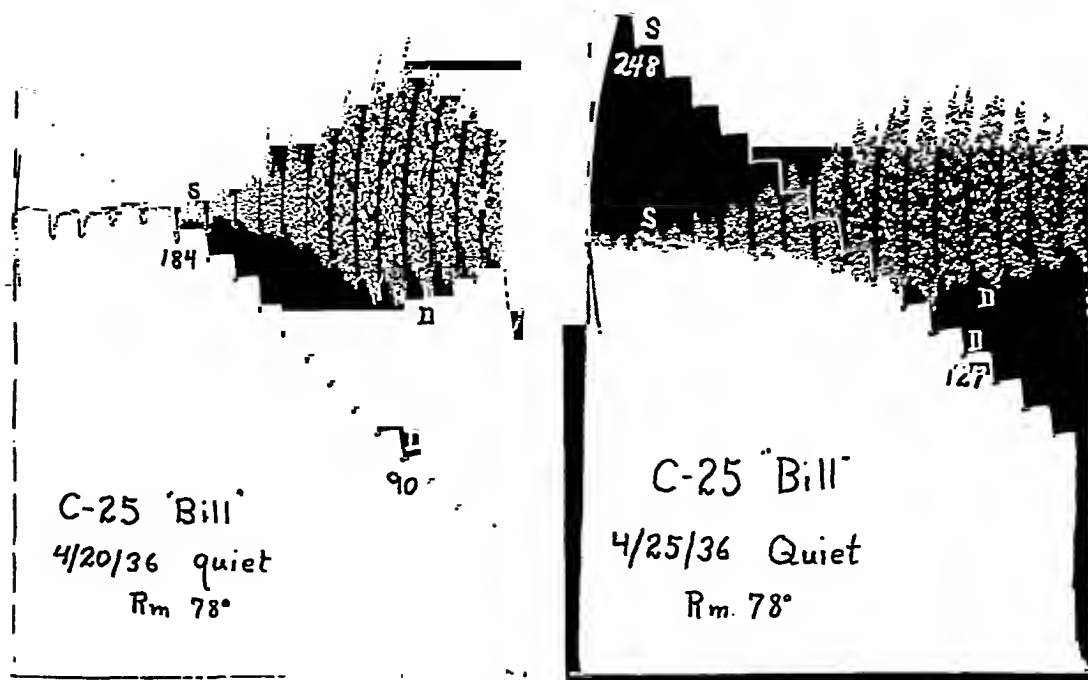


FIG. 2. TYPICAL SPHYGMOGRAPHIC RECORD OF BLOOD PRESSURE ON DOG C-25.

Tracing April 20, 1936, taken five months after application of Goldblatt clamp to right renal artery.  
Tracing April 25, 1936, taken three days after application of Goldblatt clamp to left renal artery.  
S—Systolic, D—Diastolic blood pressure level.

and deflation of the cuff. The grease with which the valve is kept sealed frequently is inadequate for the purpose or partially obstructs one of the openings and prevents the ready adjustment of pressure within the bell on the two sides of the rubber membrane. Any interference with this mechanism will, of course, produce bizarre paroxysms of the lever. All values on the tracings have been charted even from the first day of training, unless some obvious mechanical error occurred. Generally speaking, we have found greater variation in the systolic pressures, while diastolic readings are less labile.

Two sphygmographic records are shown in Figure 2. The first sudden increase in amplitude of the sphygmographic lever, with slight spreading of the limbs, represents the systolic pressure and sudden decrease of amplitude, the diastolic. Opinion as to appearance of systolic excursions may occasionally vary slightly (8 to 10 mm.) but the diastolic readings are nearly always sharp and unmistakable. Four or five pressures, both systolic and diastolic, have been recorded on one ordinary smoked kymograph paper, fixed and filed as a record.

#### EFFECT OF RENAL ISCHEMIA (GOLDBLATT CLAMP) ON BLOOD PRESSURE

Goldblatt and his coworkers (1) found that constriction of one renal artery almost invariably

produced hypertension with a gradual return toward the normal level while constriction of both renal arteries led to a sustained increase in blood pressure. Animals were observed for as long as fifteen months after operation.

Our own experience with this method<sup>1</sup> is reported in the following experiments.

*Experiment 1, Dog C-1 (Figure 3).* Goldblatt clamps—both renal arteries. Male, hound, weight 40 pounds. Preoperative observations of blood pressure were made in Dog C-1 from September 19, 1933, until November 28, 1933, when, following the usual retroperitoneal approach, a Goldblatt silver clamp was applied to the right renal artery. The clamp was tightened one and three-quarter turns, or three quarter turns back from complete obliteration. A prompt recovery with no rise in blood pressure ensued.

On January 3, 1934, a Goldblatt clamp was applied to the left renal artery. This time the clamp was tightened two and one-quarter turns, almost obliterating the left renal artery; faint pulsations could still be felt, however,

<sup>1</sup> Silver clamps and instruments for their application were obtained through courtesy of Dr. Harry Goldblatt, Cleveland, Ohio.



on the renal side of clamp. A sharp rise in blood pressure, particularly diastolic, followed (Figure 3).

Striking polyuria and marked nitrogen retention also occurred, blood nonprotein nitrogen rising to 182 mgm. per 100 cc. just before death. Animal lost appetite, weight and strength, and died February 3, 1934. Autopsy confirmed position of clamps.

During the four months prior to constriction of the renal artery the blood pressures in this animal showed a mean systolic value of approximately 170 mm. Hg with a steadier diastolic level with a mean of approx-

imately 70 mm. Hg. Constriction of the right renal artery caused little change, but subsequent constriction of the left renal artery brought about a sharp rise of blood pressure to a mean systolic of 200 mm. Hg and a mean diastolic of 110 mm. Hg. That the reduction of blood flow to the kidney was severe enough to cause marked renal insufficiency is attested by the rising non-protein nitrogen in the blood, increased urinary output over water intake in twenty-four hour tests, falling urinary specific gravity, cachexia, and death of the animal.

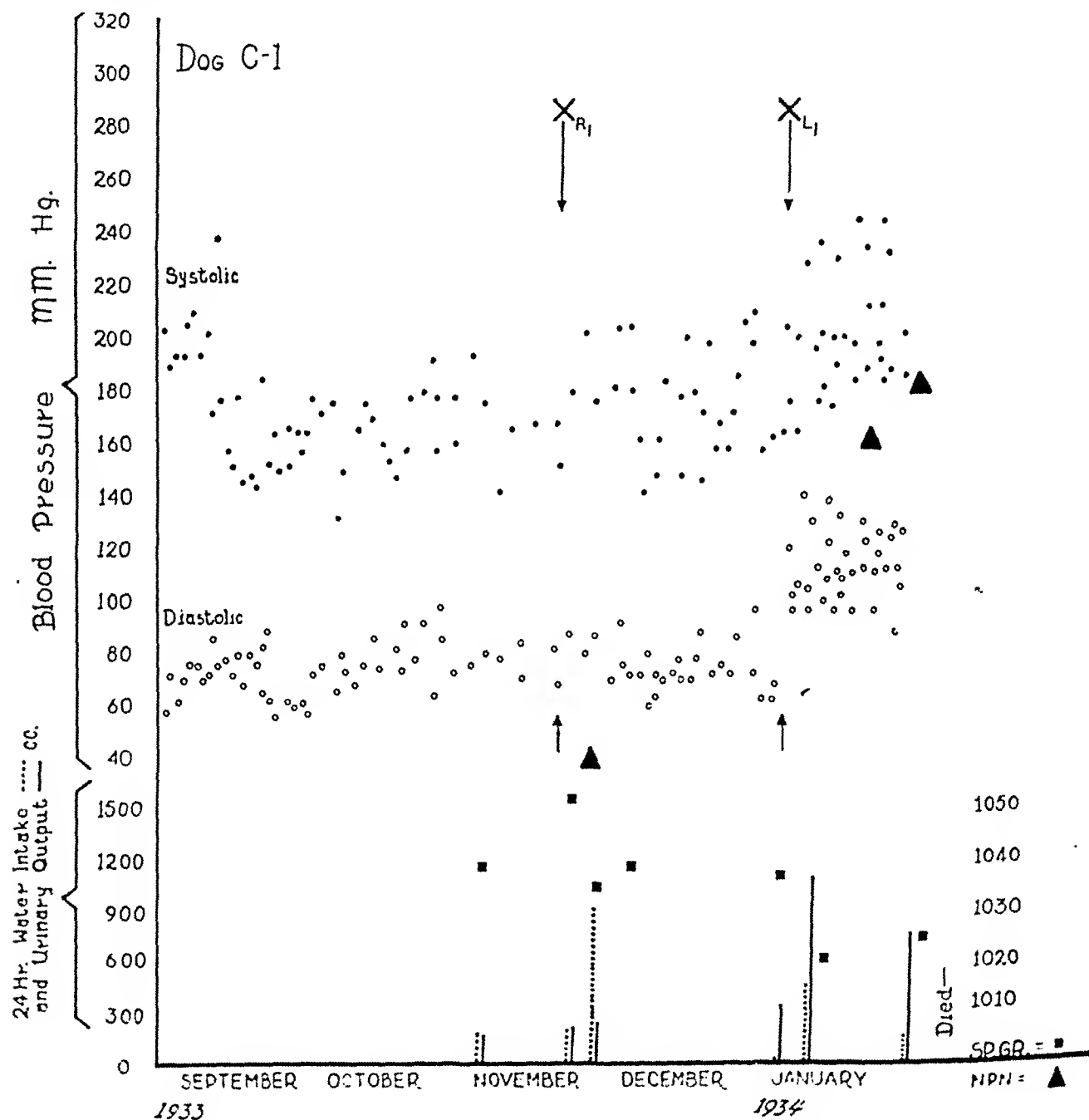


FIG. 3. GOLDBLATT CLAMPS ON RIGHT RENAL ARTERY,  $X_{R1}$ , MODERATE CONSTRICTION, AND LEFT RENAL ARTERY,  $X_{L1}$ , MARKED CONSTRICTION.

In this and subsequent charts black dots represent systolic and open circles diastolic pressure; triangle, blood nonprotein nitrogen expressed in mgm. per 100 cc.; squares urinary specific gravity; interrupted line (-----) twenty-four hour water intake; and solid line (—) twenty-four hour urinary output; arrow, time of operation; procedure.

While this discussion does not plan to deal with the pathological findings it is interesting to note that the differential ventricular dissection of this dog's heart showed a  $L/R$  ratio of 2.125 (left ventricular weight 59.4 grams, right ventricular weight 27.95 grams), a figure obtained after the method of Herrmann (8) and well above his maximum normal ratio of 1.773 based upon differential dissection of 200 hearts from normal dogs. Our own experience with the differential ventricular dissection of normal dogs has always fallen within Herrmann's  $L/R$  ratio extremes of a minimum of 1.153 and a maximum of 1.773.

The following experiment illustrates the effect of slight to moderate reduction of blood flow through the renal arteries.

pressure, particularly the diastolic, rose sharply, and then returned gradually toward more normal levels. A Goldblatt clamp was then applied to the left renal artery on February 27, 1934. Another slight rise of blood pressure was obtained; this was followed in time by a temporary fall but later a definitely elevated mean diastolic pressure. On November 8, 1935, a third operation exposed the silver clamp on the left renal artery, and this was tightened an additional two turns, almost obliterating the artery. The animal has remained in relatively good health. The absence of renal insufficiency is probably due to the fact that the renal arteries are small and the degree of clamping indicated is not as effective as in larger dogs. Present weight is 22.5 pounds.

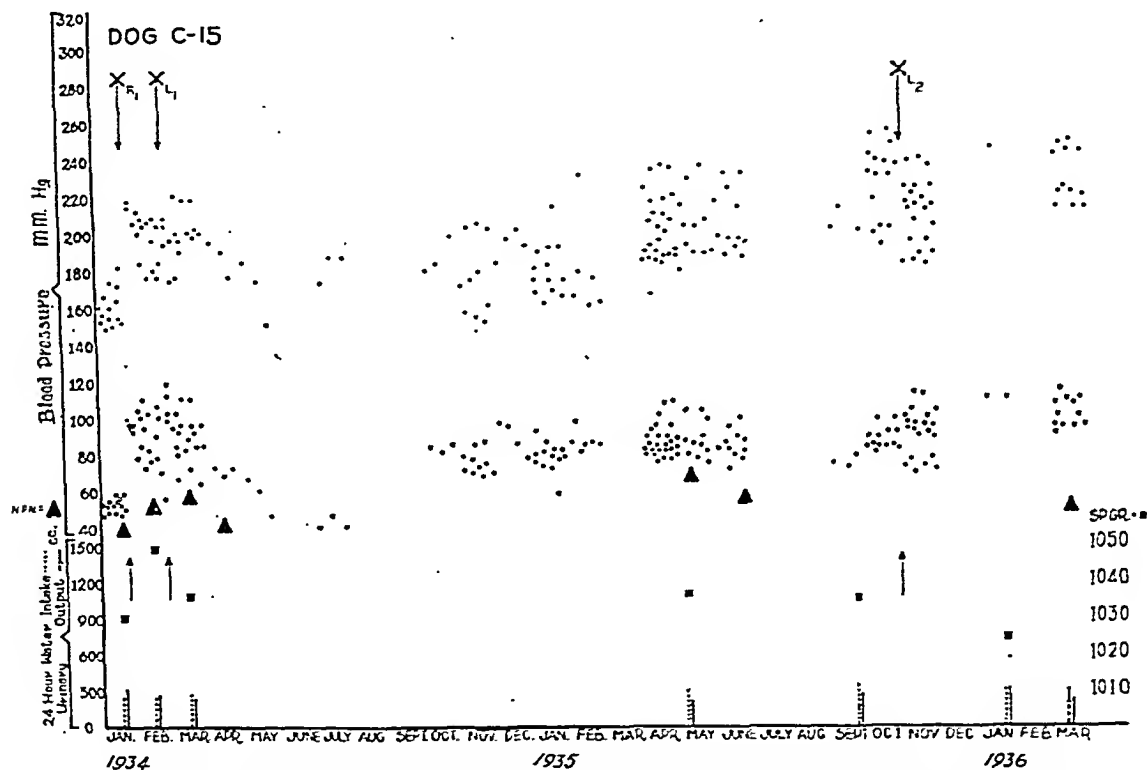


FIG. 4. GOLDBLATT CLAMPS,  $XR_1$  MODERATE CONSTRICTION RIGHT RENAL ARTERY,  $XL_1$  SLIGHT CONSTRICTION LEFT RENAL ARTERY,  $XL_2$  ADDITIONAL AND MARKED CONSTRICTION OF LEFT RENAL ARTERY.

*Experiment 2, Dog C-15 (Figure 4).* Goldblatt clamps—both renal arteries. Male, mongrel, weight 21.5 pounds. Control observation for preoperative levels of blood pressure were made in this animal from January 11 to January 25, 1934. The diastolic pressure was low and unusually stable during the control period. On January 25, following the usual retroperitoneal approach, a Goldblatt clamp was applied on the right renal artery, tightened to the point of complete obliteration, and then loosened one-half turn, allowing a small amount of blood to pass through the artery to the kidney. The blood

This dog, with less severe constriction of the renal arteries, has shown slight but definite hypertension but no diminished renal function. During the control period prior to operation the mean systolic pressure was approximately 160 mm. Hg, and the diastolic 52 mm. Hg. For months following moderate constriction of the renal arteries the systolic pressure approximated a mean of 185 mm. Hg and the diastolic a mean of 80 mm. Hg. Variations occurred but the tendency, except on two occasions, has been toward the maintenance of relatively higher diastolic pressure. This slight but definite rise in blood

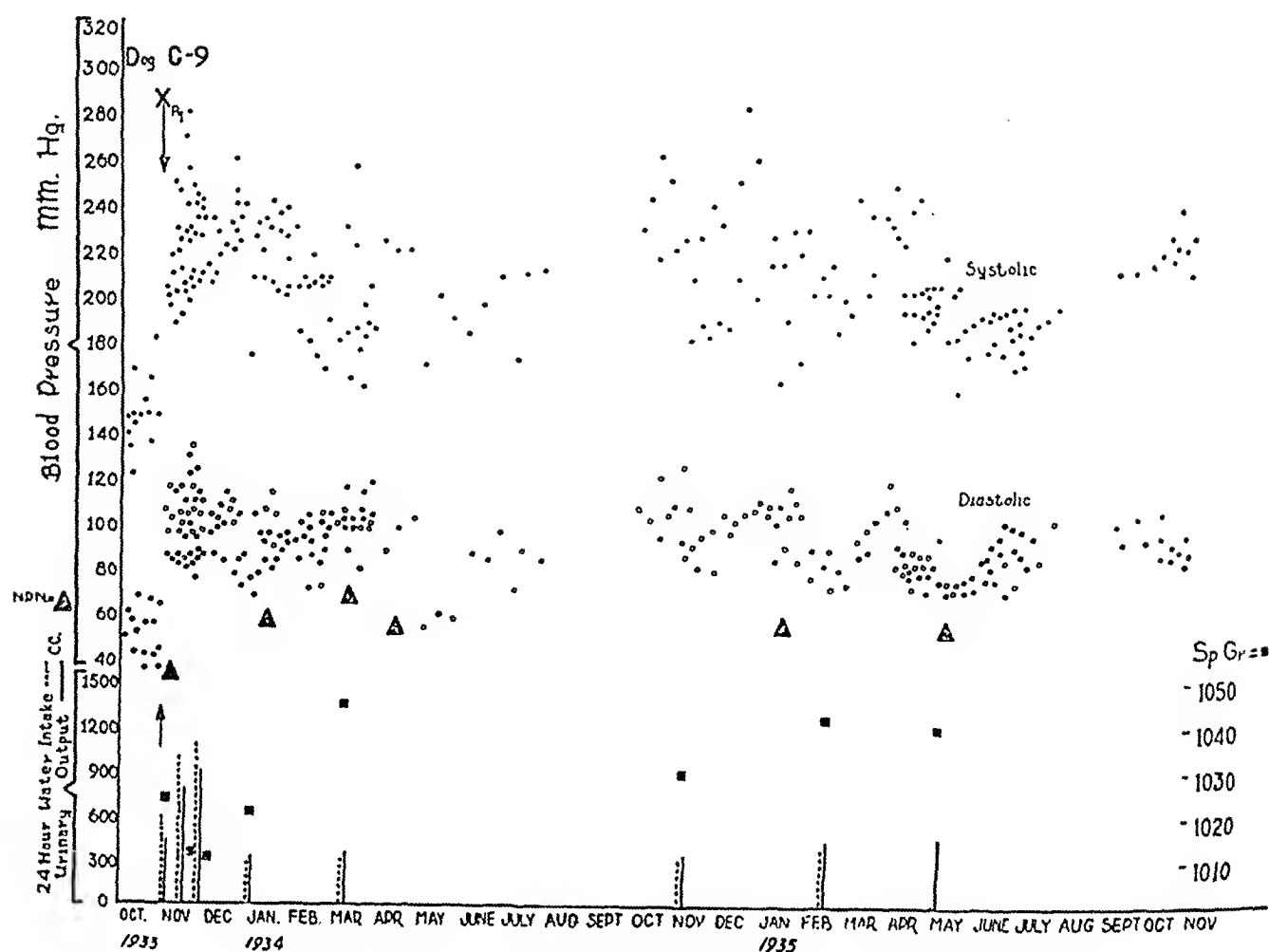


FIG. 5. GOLDBLATT CLAMP,  $XR_1$ , MARKED CONSTRICTION OF RIGHT RENAL ARTERY.

pressure, while within the limits of normal blood pressure for some animals, apparently represents mild hypertension for this dog with a greater degree of diastolic pressure developing after the third operation (Figure 4).

The third animal in this series has proved of unusual interest.

*Experiment 3, Dog C-9 (Figure 5).* Goldblatt clamp—right renal artery. Male, hound, 27 pounds. Control readings of the blood pressure were observed in Dog C-9 from October 12, to November 2, 1933. The animal was somewhat nervous, requiring patience and care during the recording of each set of blood pressures. On November 2, 1933, a Goldblatt clamp was applied to the right renal artery and tightened two and one-eighth turns, causing marked partial obliteration of the artery. There was a sharp rise in blood pressure, both systolic and diastolic, with a very gradual return toward the normal. As the pressures remained definitely above the original levels no second operation has been attempted. No nitrogen retention, polyuria, cachexia or renal insufficiency has occurred. The animal is in good condition; weighs 37.5 pounds, 24 months after operation.

Goldblatt and his coworkers noted a moderate rise in blood pressure following constriction of one renal artery but went on to complete their experiment with reduction of blood flow in both vessels. The behavior of arterial pressure following constriction of the right renal artery

in Dog C-9 of our series led us to omit application of a second clamp. A mean systolic blood pressure of 148 mm. Hg and a mean diastolic of 54 mm. Hg in the control period was followed immediately after application of a clamp to one renal artery by a mean systolic of well above 200 mm. Hg and a diastolic well above 90 mm. Hg. Elevated values have persisted for two years.

*Experiment 4 (Figure 6).* Goldblatt clamps—one renal artery. Dogs C-9, C-18, C-24, C-25, C-29, C-30, C-31 and C-34. The unusual behavior of Dog C-9 and the fact that Goldblatt and his coworkers constricted the right renal artery first, with more striking results, led us to investigate blood pressure changes following approximately equal constriction of right and left renal arteries. Goldblatt clamps were applied in the usual fashion to the right renal artery of four dogs, the clamps tightened to complete obliteration and then loosened one-half turn. A similar procedure was carried out on three additional dogs using the left renal artery. Blood pressures were recorded several days before and after each experiment and significant rises occurred in all animals (Figure 6) save the last (C-30). Finally, thinking that trauma or edema might affect the renal pedicle, we applied a clamp to the right renal artery of one dog (C-30), tightened and immediately removed the clamp. Severe constriction of either the right or left renal artery frequently leads to a significant rise

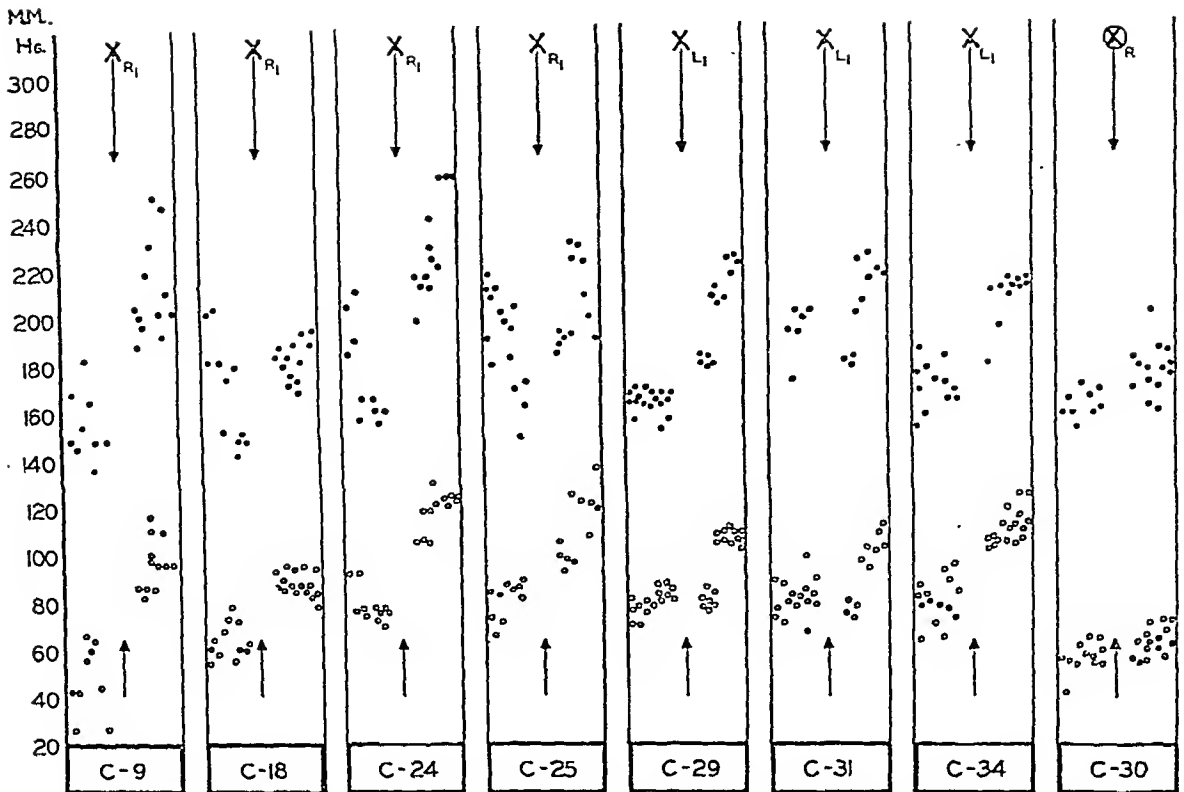


FIG. 6. GOLDBLATT CLAMPS, BLOOD PRESSURE BEFORE AND AFTER APPLICATION OF CLAMPS TO RIGHT RENAL ARTERY ( $XR_1$ ) OF FOUR DOGS AND TO LEFT RENAL ARTERY ( $XL_1$ ) OF THREE DOGS, ALL SEVEN CLAMPS ADJUSTED TO PRODUCE APPROXIMATELY SAME DEGREE OF MODERATELY SEVERE CONSTRICTION.

Dog C-30 had clamp applied to right renal artery ( $(X)R$ ) and removed at same operation.

of both systolic and diastolic pressures and does not occur following operative manipulation of the renal pedicle.

#### EFFECT OF REDUCTION OF RENAL TISSUE ON BLOOD PRESSURE

Numerous investigators have studied blood pressure in animals following the reduction of functional kidney tissue. Of the earlier experiments, those of Pässler and Heineke (9) are the most convincing. These workers reduced the kidney substance of eighteen dogs by successive operations. Seven of these dogs survived four weeks without extreme wasting. In five dogs blood pressure studies, with femoral artery readings, were made before operation, and again eight days and several weeks after the last operation. These five dogs showed an average increase in blood pressure of 21.5 mm. Hg. When too much kidney tissue was removed, according to the au-

thors, cachexia followed with a falling blood pressure. In spite of the relatively short duration of the experiments, for all seven dogs the average proportionate weight of left to right ventricle showed an increase over the normal of 28.5 per cent. Polyuria was said to have developed regularly prior to the blood pressure rise. The widely quoted studies of Rose Bradford (10), while of limited value from the standpoint of the study of hypertension, were sufficiently extensive to approximate subtotal nephrectomy for dogs. A seventy-five per cent reduction of kidney substance almost invariably led to asthenia and death of the dog in one to six weeks. Although Pearce (11) and his coworkers have been able to remove at times seventy-five per cent of renal tissue in dogs with survival, both workers have established that about one-third of the kidney substance is necessary for relatively healthy survival. Janeway (12) in a number of dogs, ex-

cised one kidney and ligated the arterial branches to the other close to the hilus, leaving infarcted renal tissue in situ. One of these dogs survived thirty-nine days after operation and showed a rise of systolic blood pressure from an average of 106 mm. Hg to 127 mm. Hg during the first twenty-one days; later the blood pressure fell, and the dog became cachectic. Only approximate systolic figures were taken by the use of a Riva-Rocci cuff on the foreleg, palpation of the artery serving as a criterion for the estimation of pressure.

Relatively recent observations on blood pressure following reduction of renal substance have been more convincing. Cash (3), using the Kolls sphygmograph, showed rises of both systolic and diastolic blood pressures in dogs after extensive ligation of renal arteries as well as following subtotal nephrectomy. These changes in pressure occurred independently of renal insufficiency and were not accompanied by changes in blood volume. The significance of these experiments was materially lessened by the short periods of observation in many instances. The elevations in pressure were interpreted as depending on two main factors, namely, a marked reduction of the normal kidney substance, plus the presence within the body of degenerating renal tissue due to inadequate blood supply. Subsequently Cash (13) observed that the blood pressure of dogs fell rapidly after bilateral nephrectomy, though a marked rise, persisting until death, occurred after complete ligation of both renal arteries. This observation was originally considered a support to the idea of a pressor substance liberated from degenerating renal tissue.

Hartman, Bolliger and Doub (14), finding renal tissue sensitive to x-ray, irradiated kidneys of large young dogs. This produced definite and marked renal insufficiency in some animals. They recorded both systolic and diastolic hypertension in several animals by the auscultatory method of Allen (15).

More recently, Chanutin and Ferris (16) have demonstrated the occurrence of hypertension and cardiac hypertrophy in rats following partial nephrectomy. They used an intra-carotid direct method for determining blood pressure with the animal under light anesthesia, partially obviating

the objection to a single terminal reading by the study of a large number of animals at various periods after subtotal nephrectomy. Wood and Ethridge (17) repeated a portion of this experiment on a similar series of rats, confirming entirely the occurrence of hypertension and also describing fatty changes in the afferent arterioles of animals surviving operation for relatively long periods.

Mark (18, 19) and Mark and Geisendörfer (20), using an auscultatory method (15, 21), recorded only negligible rises of systolic and diastolic blood pressure after marked reduction of 75 per cent of kidney tissue by excision and ligation of branches of the renal artery in dogs fed on a low protein diet. When an excessive amount of protein was given to these same dogs, a prompt rise in blood pressure followed but subsided to normal upon return to a diet low in protein. Numerous other observations concerning the metabolism, renal function and pathological anatomy of their experimental animals were made.

Contrary observations have been offered by Jensen and Apfelbach (22) who produced renal insufficiency in dogs by injecting charcoal particles in the renal artery but failed to observe arterial hypertension over a period from one week to a year. They measured blood pressure by repeated femoral puncture without anesthesia.

While the foregoing evidence favors the occurrence of hypertension following the reduction of renal tissue, obviously long time experiments with frequent observations on systolic and diastolic blood pressure on the unanesthetized dog have considerable place in further discussion. Such experiments are reported below.

*Experiment 5, Dog C-11 (Figure 7).* Partial nephrectomy. Male, mongrel, 36 pounds. This animal was under control observation from October 12 to November 7, 1933. On November 7, following retroperitoneal approach and exposure, the left kidney was exposed, a clamp placed across the lower pole, mattress sutures put in, and 10 grams of this kidney removed. Following this there was no appreciable change in blood pressure. On November 14, 1933, the remaining normal kidney was removed (weight 39 grams). The dog made an uneventful recovery, and in the following months the blood pressure showed a very slight and gradual elevation. The animal has remained in apparently good

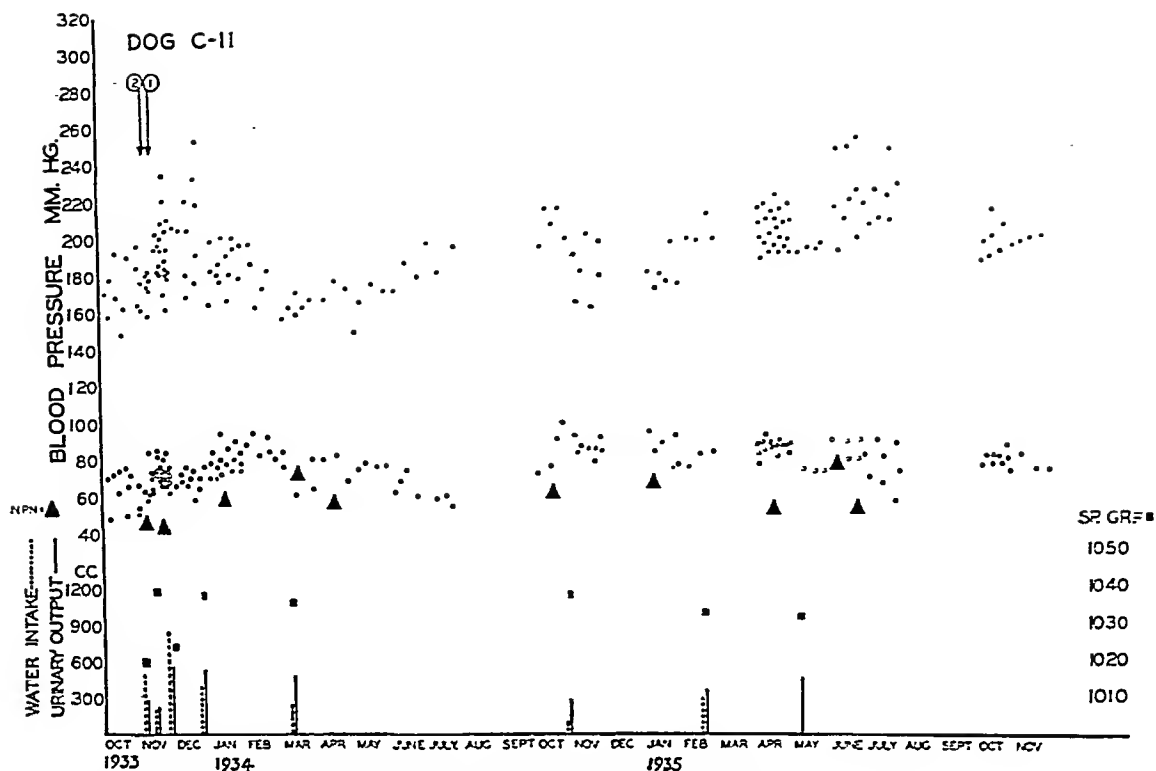


FIG. 7. PARTIAL NEPHRECTOMY, REMOVAL OF APPROXIMATELY SIXTY-FIVE PER CENT OF RENAL TISSUE.

②—Removal of portion of left kidney. ①—Removal of right kidney.

health, has no appreciable renal insufficiency, but his weight has decreased to 24 pounds.

In this animal approximately sixty-five per cent of renal tissue has been removed, allowing for partial destruction of tissue along suture lines. The approximate systolic mean blood pressure before operation was 174 mm. Hg and the diastolic mean 62 mm. Hg. In the twenty-five months following the second operation the systolic mean has varied from 161 mm. Hg to 226 mm. Hg, with a tendency to remain above 190 mm. Hg during the last ten months. Following operation, the diastolic mean has varied from 57 mm. Hg to 86 mm. Hg with a tendency to remain above 72 mm. Hg during the last twelve months. The absence of severe renal insufficiency is indicated by the relatively slight elevation of nonprotein nitrogen and relatively normal urinary specific gravity.

*Experiment 6, Dog C-2 (Figure 8).* Partial ligation of renal artery and partial nephrectomy. Male, mongrel, 30 pounds. Dog C-2 was under control observation from October 18, 1933, to December 7, 1933. On the latter date, the posterior branch of the left renal artery was ligated and one pole of the kidney removed (16 grams). The dog recovered promptly without complications. Eight days later, December 15, 1933, the remaining normal kidney was removed (weight 34 grams).

Following this the animal showed definite polyuria and nitrogen retention, the nonprotein nitrogen mounting to

160 mgm. per 100 cc. blood, later returning to values from 62 to 73 mgm. per 100 cc. blood. The dog has recovered and remains in apparently good health. His present weight is 29.5 pounds.

Approximately 75 per cent of renal tissue was removed in this animal. Nitrogen retention occurred after the second operation, with marked polyuria. Although the condition of the animal has greatly improved during subsequent months, polyuria persists with a falling urinary specific gravity. Most striking have been the wide fluctuations of systolic pressure and the relatively steady diastolic blood pressure; although a slight definite trend upward occurs in each, the most impressive change has taken place in the diastolic pressure. The mean systolic blood pressure before operation was 169 mm. Hg and the mean diastolic 78 mm. Hg. After the second operation the mean systolic pressure rose to remain consistently above 185 mm. Hg, with the exception of one of the last ten months, while the diastolic mean remained above 90 mm. Hg, and generally above 100 mm. Hg, for the same period.

*Experiment 7, Dog C-6 (Figure 9).* Partial ligation of renal artery without removal of renal tissue. Male, terrier, 25 pounds. Dog C-6 was observed during a control period from October 12, 1933, to January 16, 1934. On the latter date, the left renal artery was well exposed and showed the usual anterior and posterior

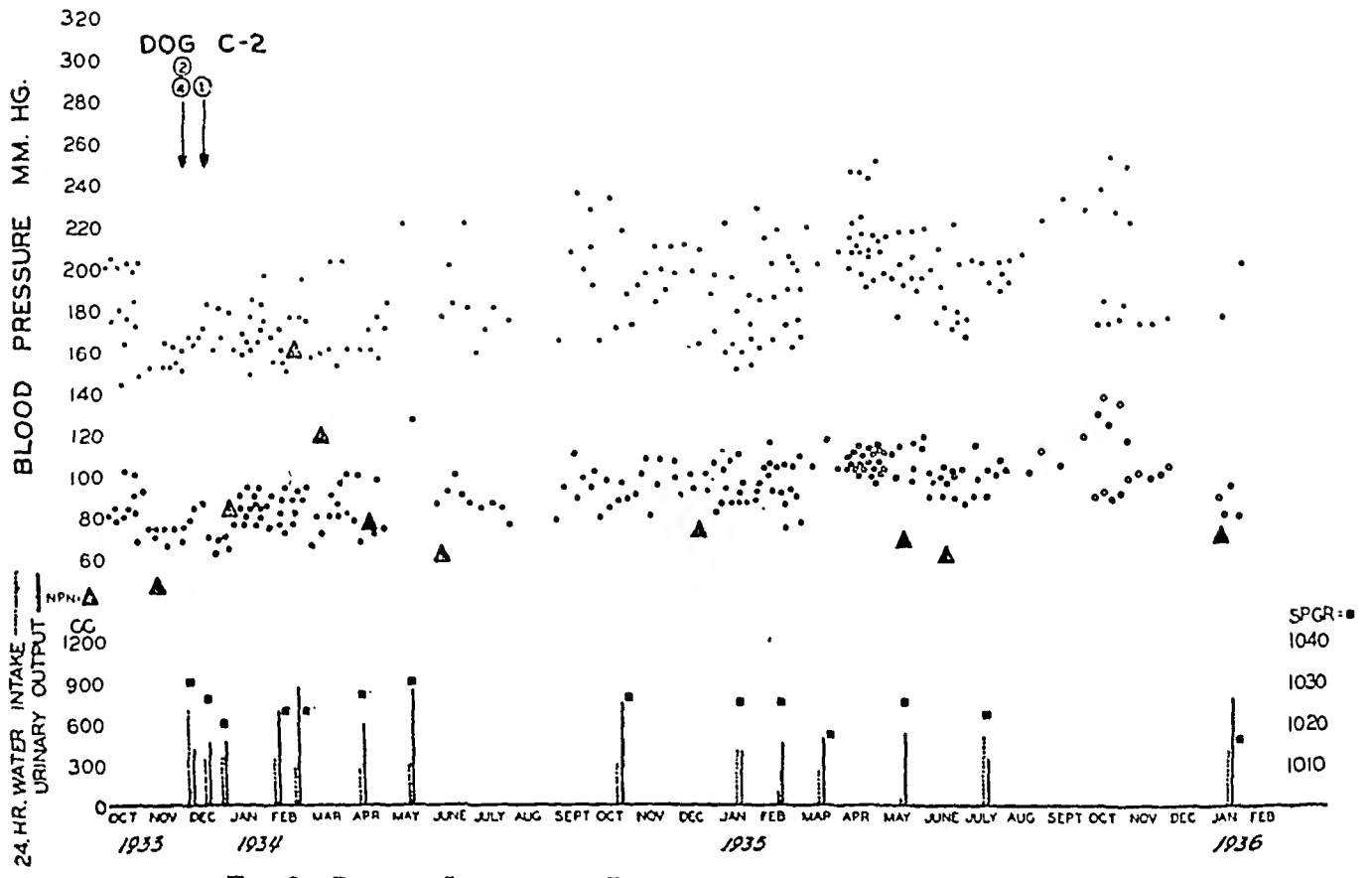


FIG. 8. PARTIAL LIGATION OF RENAL ARTERY AND PARTIAL NEPHRECTOMY.

(4)—Ligation of posterior branch of left renal artery. (2)—Removal of portion of left kidney. (1)—Removal of right kidney.

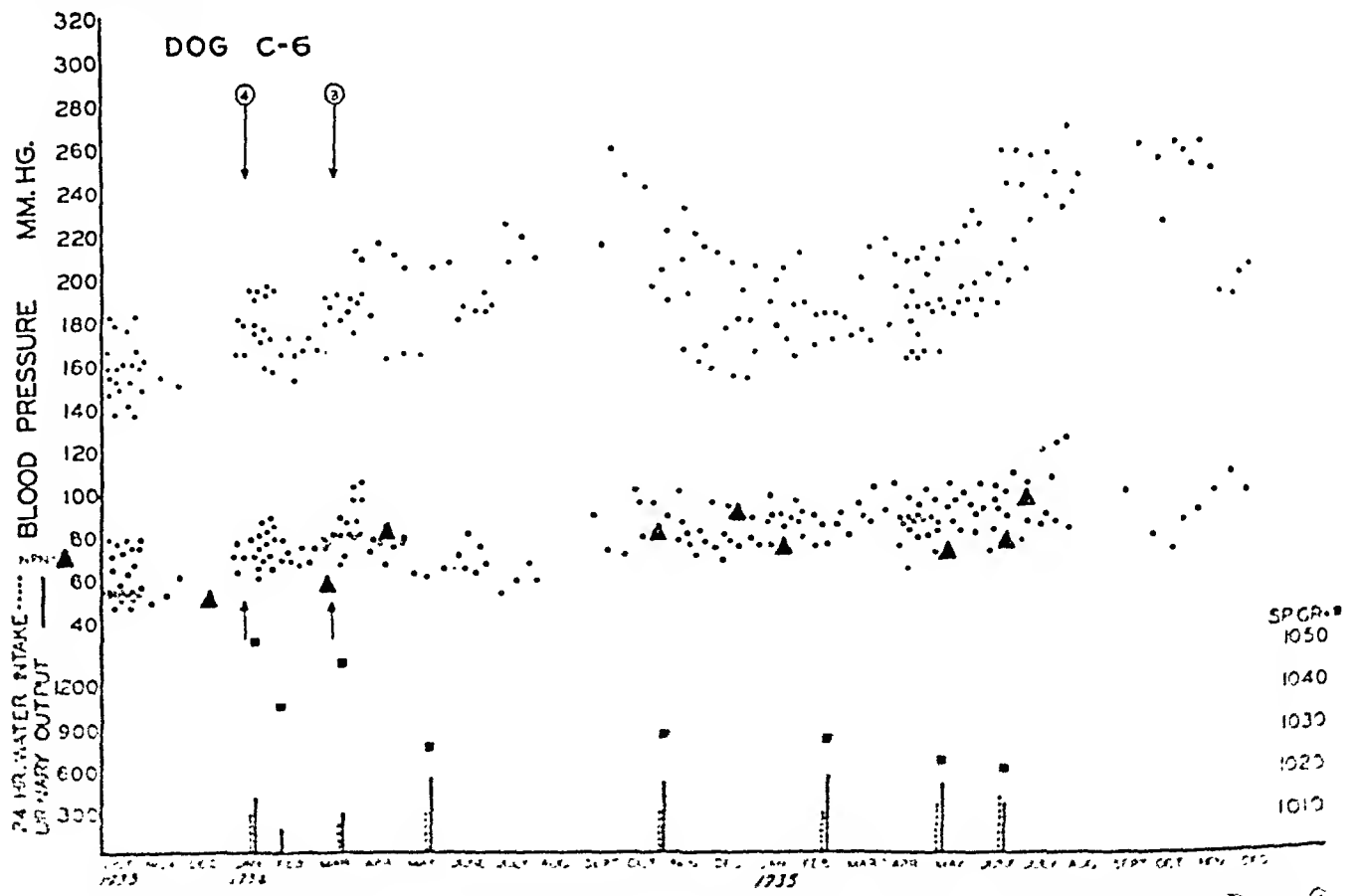


FIG. 9. PARTIAL LIGATION OF RENAL ARTERY, LEFT (4), AND COMPLETE LIGATION OF RENAL ARTERY, RIGHT (1).

branches. Close to the hilum of the kidney the posterior branch further divided into two small arteries. The anterior and one small division of the posterior branch were ligated, leaving only one small division of the posterior branch intact. Following this there was little change in the blood pressure level.

On March 14, 1934, the arterial supply to the right kidney was exposed in the usual fashion and the ligature placed around the anterior and posterior branches, obliterating the blood supply to this kidney, and leaving the dog with approximately 25 per cent of his original renal tissue.

Following these procedures there was slight but definite nitrogen retention, and very slight but definite gradual increase in the diastolic pressure. The weight of the animal at present is 26.5 pounds.

The reduction of kidney substance in this experiment approaches the desired result. A low grade renal insufficiency has been produced as shown by a slight but definite nitrogen retention in the blood, polyuria, and a gradually decreasing specific gravity of the urine, in twenty-four hour tests. A slow but definite rise in diastolic and systolic blood pressure has been recorded (Figure 9). Fluctuations of systolic blood pressure occur in wide swings but a general upward trend occurs from the preoperative mean systolic level of 164 mm. Hg and mean diastolic of 61 mm. Hg. Definite rises of both

systolic and diastolic pressure occurred after each operation, and in the twenty-one months following the second operation the monthly systolic mean has ranged from 176 to 256 mm. Hg. The less variable diastolic pressure has also shown a steady rise with a monthly diastolic mean of 59 to 103 mm. Hg.

*Experiment 8, Dog C-3 (Figure 10).* Partial ligation of renal artery without removal of renal tissue. Male, shepherd, 40 pounds. Control blood pressure readings were made in Dog C-3 from October 4, 1933, to December 5, 1933. On the last date, following the usual retroperitoneal approach, the right renal artery was exposed and ligated, cutting off all blood supply to the kidney. No kidney tissue was removed. No change in blood pressure followed this operation. On December 12, 1933, the artery to the left kidney was exposed and found to have the usual anterior and posterior branches. The posterior branch was ligated.

The animal remains in good condition without definite nitrogen retention, weight 40 pounds.

A variation in method here seemed to influence the blood pressure response rather definitely. Contrary to the usual procedure of removing intact kidney at the second operation, thereby allowing edema and injury from trauma to subside in the small remaining stump, the right renal artery was completely ligated at the first operation and partial ligations of left renal artery

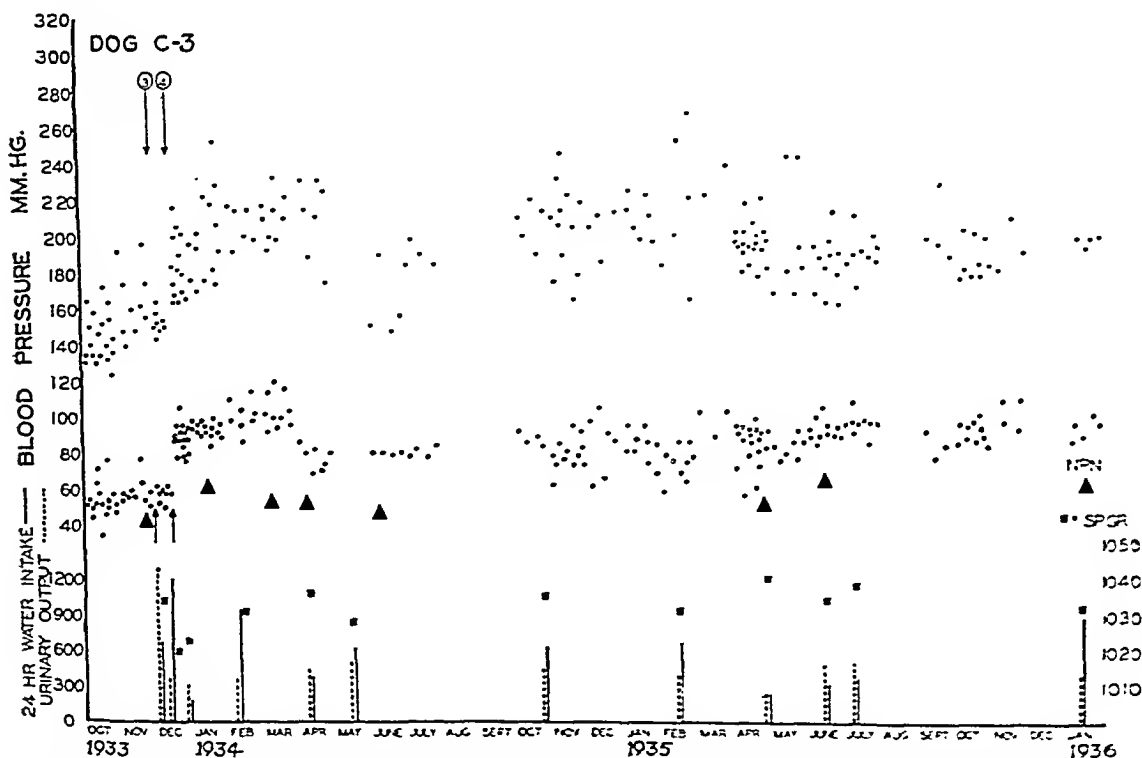


FIG. 10. PARTIAL LIGATION OF RENAL ARTERY WITHOUT REMOVAL OF RENAL TISSUE, COMPLETE LIGATION OF RIGHT RENAL ARTERY ③ AND LIGATION OF POSTERIOR BRANCH OF LEFT RENAL ARTERY ④.



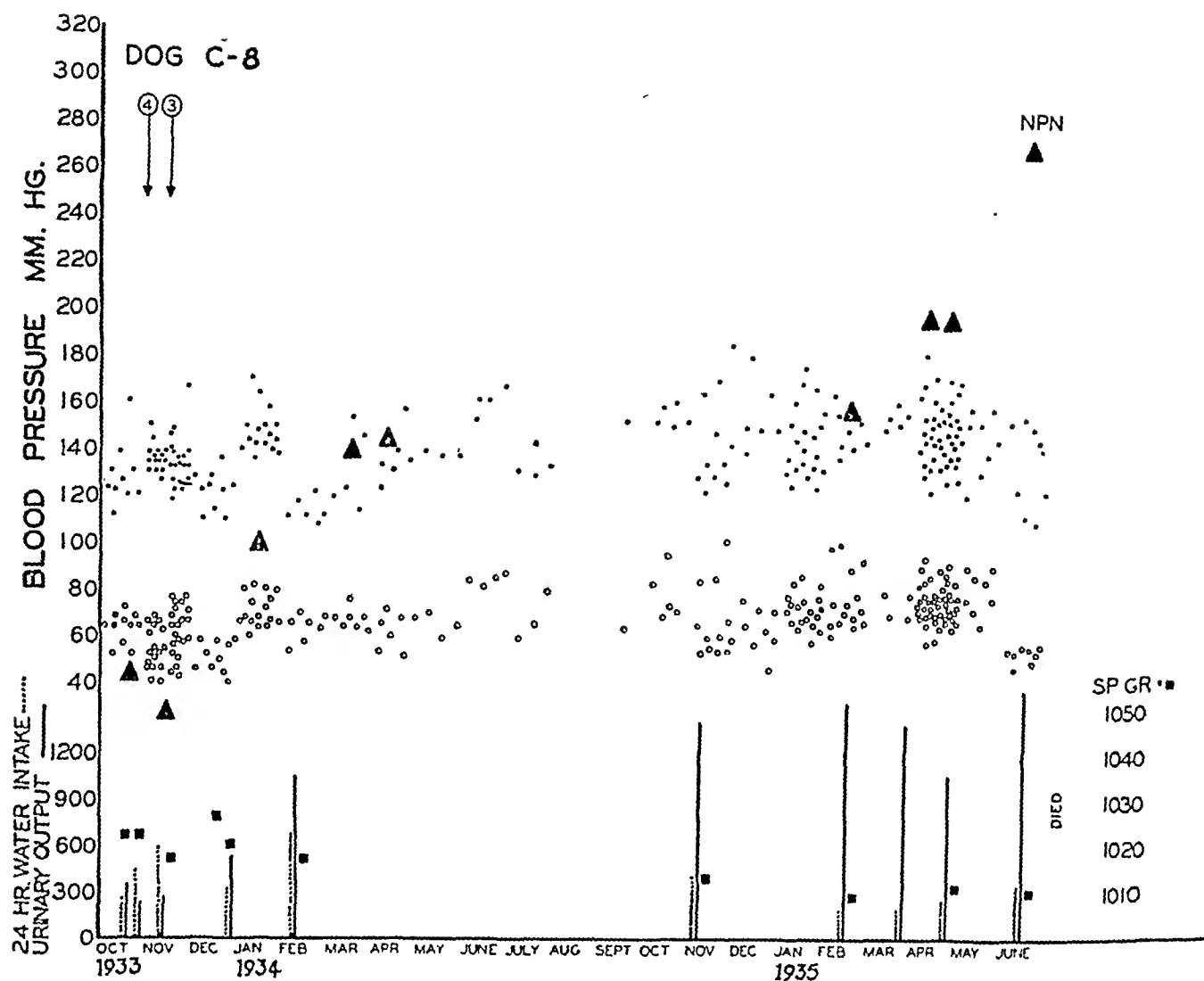


FIG. 11. PARTIAL LIGATION OF RENAL ARTERY WITHOUT REMOVAL OF RENAL TISSUE.

④ Ligation of posterior branches of left renal artery, and ③ ligation of right renal artery.

branches were done at the second operation, no tissue being removed at any time. A sharp and prompt rise of blood pressure followed the second operation, as shown in Figure 10. The mean blood pressures prior to the second operation were approximately 154 mm. Hg systolic and 57 mm. Hg diastolic, whereas immediately after operation and for three and one-half months thereafter the monthly mean systolic blood pressure rose to 183 to 210 mm. Hg and the diastolic to 77 to 101 mm. Hg. The pressures then decreased to somewhat lower levels but definite hypertension has continued to the present time.

Definite polyuria and fall in urinary specific gravity followed the second operation. The disappearance of the polyuria and the apparent ability of the kidney to concentrate during the hot summer months of June and July, 1935, argues for a hypertrophy of the renal stump and some recovery of function. This animal, with the exception of a short period after the second operation, has remained lively and vigorous.

*Experiment 9, Dog C-8 (Figure 11).* Partial ligation of renal artery without removal of renal tissue. Male, born April 31, 1934. Dog C-8 was derived from

October 12 to October 31, 1933, when the anterior and posterior branches of the left renal artery were exposed. The latter was found to have two divisions and these were ligated. The animal recovered promptly without elevation of blood pressure. On November 9, 1933, the main artery of the right kidney was exposed and ligated. No kidney tissue was removed at either of the above operations.

The animal remained lively for a while, but the blood nonprotein nitrogen gradually mounted from a preoperative level of 44 mgm. per 100 cc. to 275 mgm. per 100 cc. just before death. Polyuria occurred to an extreme degree, urinary output being four times as great as water intake in several tests. The appetite became poor and the weight gradually decreased to 27.5 pounds just prior to death. Death occurred on June 14, 1935.

Following the second operation, the remaining renal tissue (about twenty-five per cent of the total) showed inadequate renal function as evidenced by a steadily rising nonprotein nitrogen in the blood, marked polyuria and falling urinary specific gravity, and the animal finally died with the typical uremic triad of symptoms: nitrogen

retention, polyuria, wasting and a terminal fall in blood pressure. Notwithstanding this marked grade of renal insufficiency, only a very slight rise in diastolic blood pressure took place in the ensuing months. That the slight rise of diastolic blood pressure was significant is suggested by the differential ventricular dissection of this dog's heart at autopsy. The *L/R* ratio of 2.009 (left ventricular weight 42.00 grams, right ventricular weight 20.90 grams) is sufficiently above the extreme normal figure of Herrmann (8) to offer additional evidence that low grade hypertension may lead to left ventricular hypertrophy.

#### COMMENT

The foregoing experiments record the blood pressure findings in dogs subjected to interference with renal substance or circulation for short and long periods. Much could have been done to smooth out the variations in the blood pressure curves reported if the figures for a single day had been reduced to a single mean value. However, the use of all individual values regardless of variations from the apparent levels at the time, has brought out beautifully the value of trends, particularly in the diastolic blood pressure. The relative stability of the diastolic pressure is especially well illustrated by Dog C-1 (Figure 3). This animal was particularly quiet, showing no effect of excitement except in the first few days of training. The diastolic rise following production of renal ischemia was much more pronounced than the systolic. Renal insufficiency, hypertension and left ventricular hypertrophy in this animal were unmistakably present.

That a slight diastolic trend upward from the preoperative level may represent definite hypertension seems to be indicated by a study of Dog C-8 (Figure 11). Following marked reduction of renal tissue, definite renal insufficiency promptly followed, lasted for eighteen months, and finally ended in typical uremic death. While the blood pressure changes following operation in this animal were not marked, we believe an upward trend in diastolic pressure was definite, except in the last few days during uremia. A small but definite left ventricular hypertrophy attested to the probability of hypertension. The method, therefore, appears valuable for the measurement of small as well as marked changes in blood pressure.

The contention of some observers, notably Jensen and Apfellbach (22) that unanesthetized dogs

should be retrained after any temporary omission of observations of blood pressure is certainly true. However, in our experiments, hypertension was considered present only when values exceeded the pressures of the early training period. The trends became quite convincing when it was seen that the blood pressure was rising in spite of the fact that the animal was becoming progressively better trained. Here again, lack of training is generally reflected more violently in the systolic than diastolic pressures (Figures 5, 9, 10). More important, however, is the natural reaction of the dog. For example, Dogs C-1 (Figure 3) and C-8 (Figure 11) proved to be unusually quiet from the beginning, and omission of all studies during the eleventh month in Dog C-8 was followed by a slight elevation of systolic but no change of diastolic values. On the other hand, Dog C-9 (Figure 5), following a two month vacation (eleventh and twelfth months), definitely needed retraining as shown by a sharp elevation of systolic pressure; there was, however, much less disturbance of diastolic pressure. The dog should be comfortably warm to avoid fluctuations of blood pressure. We have therefore been careful to avoid low room temperatures when recording blood pressures. The trends of blood pressure in our animals generally seemed to be lower in summer.

Our observations indicate that varying degrees of hypertension follow reduction of renal tissue by the methods outlined above. Exact knowledge as to why this occurs is lacking, for the rise in blood pressure is not proportionate to the degree of renal insufficiency, nor does marked hypertension necessarily accompany even the severest renal insufficiency (Dog C-8, Figure 11). Conversely, hypertension may be produced experimentally in dogs by renal ischemia without material decrease in renal function (Goldblatt et al. (1)). The conclusion apparently follows that although hypertension is in some way related to the reduction or alteration of renal tissue it is largely independent of renal function.

The approach to the problem of experimental hypertension has been greatly facilitated by the renal artery clamp method of Goldblatt et al. (1). Reduction of renal tissue by arterial ligation or partial nephrectomy may or may not bring about

hypertension. The renal ischemia method invariably does lead to elevation of the blood pressure. However, much remains to be explained concerning the physiology of renal ischemia hypertension. Apparently the occurrence of renal ischemia hypertension is not prevented by renal denervation (24) or excision of splanchnic nerves (23). However, a number of our own dogs (Figure 6) as well as some of the animals of Goldblatt and his coworkers exhibited hypertension following the partial clamping of one renal artery. This phenomenon is lacking a patent explanation if a nervous mechanism is excluded. Further study of the rise in blood pressure following ischemia of one kidney would seem to be indicated. As Goldblatt et al. (1) have already suggested, an indirect humoral mechanism may be possible, but no good evidence of this is so far available.

#### SUMMARY AND CONCLUSIONS

1. A modification in the sphygmographic assembly of Kolls which has proven highly satisfactory for recording both the systolic and diastolic blood pressure of unanesthetized dogs has been reported.

2. Studies of the blood pressure and gross renal function of eight dogs subjected to various degrees of renal ischemia, to subtotal nephrectomy and to ligation of renal arteries are submitted. Elevations of both systolic and diastolic blood pressure have been observed for periods of from two and one-half to twenty-four months.

3. Seven additional short time experiments in dogs demonstrated that severe constriction of one renal artery, either the right or the left, leads to a significant rise of both systolic and diastolic blood pressures.

4. Of several methods hitherto used to produce sustained arterial hypertension in dogs, renal ischemia, as accomplished by the Goldblatt clamp, has proven to be the most reliable and effective procedure.

5. No explanation of the exact mechanism by which these rises in blood pressure occurred is offered but it is our belief that hypertension following subtotal nephrectomy, ligation of renal arteries, as well as renal ischemia, probably results from the same fundamental cause.

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# RETICULOCYTOSIS IN THE GUINEA PIG FOLLOWING INJECTIONS OF GASTRIC JUICE AND CONGO RED<sup>1</sup>

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Various investigators have stated that liver extracts, effective in the treatment of pernicious anemia, cause a rise in the number of reticulocytes in the guinea pig (1, 2, 3, 4). The route of administration (intraperitoneal, subcutaneous, oral) does not affect the type of response nor the height of the reticulocyte rise, but it appears to change slightly the time of the peak of the reticulocytosis. Jacobson (2) defines a positive response as "a rise of the reticulocyte count to at least 2.0 per cent on two successive days, within 6 days after the administration of the substance to be tested." According to his data it falls on the 4th to 7th day following the injection. All the substances tested by him were administered intraperitoneally. Landsberg and Thompson (3) who daily injected commercial liver extract subcutaneously in the abdominal wall obtained a reticulocytosis with peaks between the 5th and 11th days following the beginning of the treatment, most of them falling on the 8th day or later. Miller and Rhoads (4), administering liver extract orally, found the maximum reticulocytosis occurred between the 7th and 12th days. These data indicate that the anti-anemic substance, when injected intraperitoneally, produced a reticulocyte peak at an earlier time than when given either subcutaneously in the abdominal wall or orally.

Liver extracts are not the only substances which cause this reticulocytosis in guinea pigs. Miller and Rhoads (4) found that oral administration of extracts of rice polishings and vegex, when incubated with gastric juice, will also give a similar response. The same is also true of gastric juice incubated with beef muscle, with or without pepsin, provided the gastric juice is not heated to 90° C. before intraperitoneal administration (2).

The following report deals with the reticulocyte response of guinea pigs to the intramuscular administration of gastric juice from patients with

or without pernicious anemia, as well as the response to Congo red and to human gastric juice given intraperitoneally.

## METHOD

Daily reticulocyte counts were made from the blood obtained from an ear vessel of the guinea pig. The blood was received on the corner of a clean number 1 cover slip, and inverted on a slide on which there was an aqueous solution of 1 per cent brilliant cresyl blue in 0.5 per cent sodium citrate and 0.9 per cent sodium chloride. The blood and stain were mixed, the cover slip rimmed with vaseline, and the preparation allowed to stand at least 45 minutes before the reticulocytes were counted. The red cells were examined under oil immersion, at least 1,000 cells being counted in various parts of the cover slip, and the percentage of reticulocytes calculated.

All the material to be tested was introduced by intramuscular injection in a single dose into the thigh of the test animal. The only exceptions to this were the few intraperitoneal injections made of normal gastric juice and of Congo red.

## RESULTS

### 1. Control series

(A) Daily counts were made on 6 non-injected guinea pigs. The average percentage of the reticulocytes varied from 0.85 to 1.62 per cent of the erythrocytes, most of the values falling between 0.9 and 1.2 per cent (Figure 1 and Table I). The greatest change in the reticulocytes was an increase of 90 per cent (limits 0 to 103 per cent) over the lowest value.

(B) Liebig's beef extract was used as a control injection. A solution of the extract was made in distilled water so that 1 cc. of the solution represented 0.5 gram of the original material. This amount was injected intramuscularly into 4 guinea pigs. Daily counts for eight consecutive

<sup>1</sup> Supported in part by a grant from the Rockefeller Fluid Research Fund.

TABLE I

*Reticulocyte level in uninjected normal guinea pigs on successive days*

Guinea pig number	Reticulocyte (per cent of R.B.C.)											Day of peak	In-crease
	Days												
	1	2	3	4	5	6	7	8	9	10	11		
531	1.2	1.0	0.5		0.5	0.6	0.4	0.7	0.6	0.8		0	0
532	0.4	0.7	0.9		1.3	1.0	0.7	0.4	0.5	1.0		4	103
533	1.4	2.2	2.0		0.8	2.5	2.0	0.8	1.7	1.3	1.0	5	11
534	1.8	1.5	0.9		1.2	1.8	1.5	0.7	0.6	0.5	0.8	0-5	0
537	1.0		1.3	1.9	1.1	1.3	1.0	2.1	1.0	2.0	1.5	7	110
540	1.6	2.2	1.2	1.4		1.0	1.0	0.7	0.8			1	0
Average	1.23	1.52	1.13	1.62	.98	1.36	1.1	.90	.85	1.12	1.1	3	90

TABLE II

*Reticulocyte response in guinea pigs following intramuscular injection of Liebig's beef extract*

Guinea pig number	Injection	Reticulocyte (per cent of R.B.C.)											Day of peak	In-crease
		Be-fore in-jection	Days after injection											
			1	2	3	4	5	6	7	8	9	10		
	grams													per cent
539	0.5	0.8	0.5	0.6		0.5	1.0	1.5		0.9		0.4	6	130
540	0.5	0.1	0.3	0.6		0.2	0.5	0.2		0.7		1.3	10	550
541	0.5	0.2	0.5	0.5		1.0	1.6	0.7		1.3		2.2	10	525
542	0.5	2.4	1.4	1.3		0.9	1.9	1.6		1.1		0.7		
Average		.87	.67	.75		.65	1.25	1.0		1.0		1.15	5	43

days showed an average reticulocyte range between 0.65 and 1.25 per cent, the latter figure being an increase of 90 per cent over the lower one (Figure 1 and Table II). Of the 2 cases where there was an increase of 500 per cent, all the values, except one, were below 2 per cent and ranged between 0.1 and 1.6 per cent; these values were very similar to those found in uninjected animals. It was concluded that Liebig's beef ex-

tract contains no substance reticulocytogenic for the guinea pig. This agrees perfectly with clinical experience, since beef extract has not been shown to possess any curative potency in pernicious anemia.

The average degree of rise of the reticulocytes following the injection of beef extract was practically the same as that found in normal uninjected guinea pigs. From these values it was decided

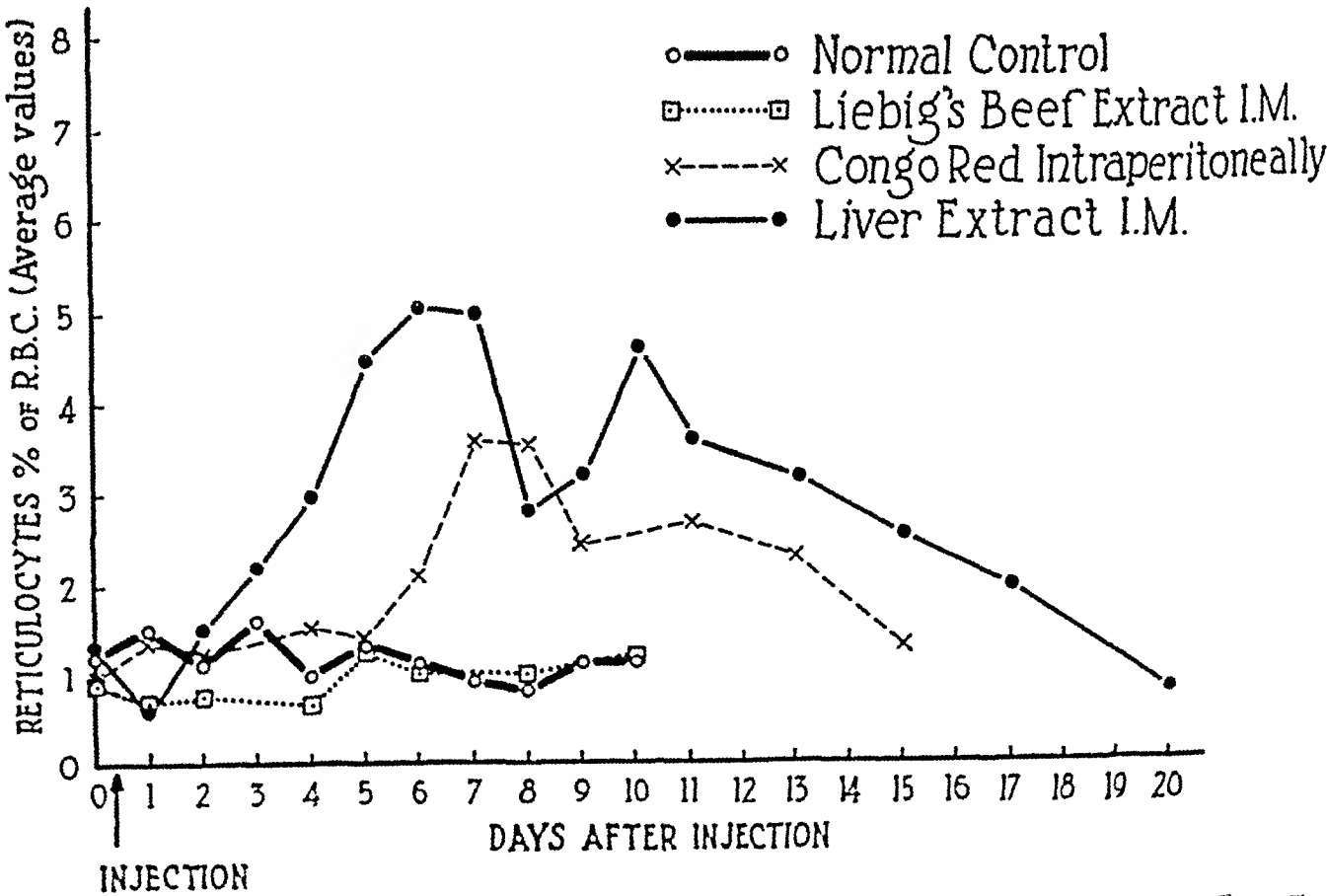


FIG. 1. RETICULOCYTE LEVEL IN UNINJECTED GUINEA PIGS AND RESPONSE TO INJECTIONS OF BEEF EXTRACT, CONGO RED, LIVER EXTRACT

that in order to be called reticulocytogenic for the guinea pig a substance must produce within eight days following the first injection an increase of the reticulocytes greater than 100 per cent of the control value, and with an absolute reticulocyte value greater than 2 per cent of the erythrocytes. Since the reaction did not begin before the 2nd day in any instance, the average of the first two counts (day of injection and first day following) was taken as the control reticulocyte value. The graphs are based on the average values obtained

from a number of animals undergoing the same treatment.

## 2. Liver extract

One-tenth cubic centimeter of commercial antipernicious anemia liver extract (equivalent to 3.3 grams of whole liver) was injected intramuscularly into the guinea pigs. About 85 per cent of the animals gave a positive response (Table III). The rise of the reticulocytes began on the second day following the injection and reached its peak

TABLE III

*Reticulocyte response in guinea pigs following intramuscular injection of antipernicious anemia liver extract*

Guinea pig number	Reticulocyte (per cent of R.B.C.)														Day of peak	In-crease
	Before injection	Days after injection														
		1	2	3	4	5	6	7	8	9	10	11	12			
															per cent	
Injection of 1 cc. Lilly L.E. (3.3 grams liver)																
531	0.9		1.4	1.7	1.4	3.0	3.8	4.2	2.0		1.0			7	366	
532	1.8		2.2	4.0	3.2	5.8	2.8	2.8	4.0		1.8			5	222	
533	1.6		1.0	0.9	1.0	5.4	2.6	2.6	2.4		0.8			5	235	
536	0.9		2.1	2.3	2.3	4.0	3.0	0.8	2.6		1.6			5	345	
541	2.3		1.8		4.0	7.2	5.0	2.6	5.4		1.2			5	213	
542	0.8		1.5	2.2	2.8	4.0	4.8	1.0	2.8		0.4			6	500	
544	1.1		1.7	1.6	2.2	3.6	3.0	3.0	2.4		1.2			5	225	
549	1.6		2.7	8.1	8.5	5.5	9.8	5.2	1.2			3.2		6	510	
550	1.3		0.7	1.6	2.0	5.0	8.4	7.4	2.8			1.4		6	550	
551	2.4		2.1	1.9	5.2	10.5	3.8	6.2	0.2			1.4		5	340	
552	1.7		0.9	4.5	4.2	5.9	3.4	5.0	2.6			2.8		5	250	
553	1.0	1.0			0.8	1.8	3.4	1.6		2.2				6	240	
554	2.3		2.5	1.0	4.6	3.6	4.8	3.6	0.2			0.8		6	109	
555	1.7		3.2	3.6	6.0	4.9	3.6	1.2	1.2			3.0		4	250	
557	2.5		1.3	1.7	1.7	1.4	5.6	4.8	0.6			2.1		7	125	
558	0.5		0.2	1.0	3.2	7.2	7.0	2.4	1.0		0.8			5	1340	
559	0.5		0.7	1.2	4.2	1.4	1.8	8.8	1.2		0.8			7	1660	
560	0.9		1.0	1.6	2.2	1.8	2.6	3.2	1.2		0.6			7	235	
561	0.6		1.0	0.8	1.0	1.6	2.4	3.6	1.0		0.2			7	500	
564	1.5		1.3	2.0	2.3	3.4	5.6	4.0	2.0			1.2		6	275	
572	1.1		1.4	3.0	2.6	7.0	7.8	5.0	2.0			2.6		6	610	
573	2.1		1.8	1.9	3.9	3.8	6.6	6.4	3.2			2.0		6	214	
575	3.4		2.7	2.1	2.5	1.8	9.8	13.0	3.0			3.0		7	280	
Injection of 0.1 cc. Lilly L.E. (3.3 grams liver)																
OBA	0.6													7	1680	
OBB	1.1	0.9	0.2	0.4	2.0	3.2	7.2	10.7	5.0			1.2		5	540	
					1.5	8.6	7.0	4.4		1.6						
Injection of 0.1 cc. Lederle (3.3 grams liver)																
1	0.1	0.5	0.9	2.7	2.8		3.2	6.3	9.4	2.5	3.7	5.0	1.7	8	3030	
3	0.1	0.1	0.3	3.0	3.2	3.2		10.0	3.6	7.4	5.1	6.1	4.3	6	9900	
4	0.1	0.2	0.3	0.5	2.0		2.9	2.5	6.0	3.0	4.1	3.5		8	3900	
Average	1.3	0.58	1.52	2.22	2.97	4.45	5.06	4.49	2.8	3.3	1.64	2.62	3.0	6	440	



on the 6th day (Individual limits of the peak 4th to 8th day). The average percentage rise was 440 per cent, ranging from 109 to 3030 per cent (Figure 1).

The ability of guinea pigs to respond to intramuscular injections of liver extract was used as a means of dividing them into two groups: reacting animals (those showing a reticulocytosis after the treatment) and non-reacting ones (those showing no reticulocytosis after treatment). Only reacting animals were used in subsequent experiments. Values obtained with 28 animals are found in Figure 1 and Table III.

When a second injection was made on the 12th to 18th day following the first one, no second reticulocytosis was obtained. This absence of a second reaction showed that a maximal effect had been obtained with the first injection, and was also used as a criterion of the activity of the materials tested.

### 3. Congo red

A 1.5 per cent solution of Congo red (Coleman and Bell), in 6 per cent glucose, was injected intraperitoneally into 6 guinea pigs. The total

TABLE IV

*Reticulocyte response in guinea pigs following intraperitoneal injection of 1.5 per cent Congo red solution*

Guinea pig number	Reticulocyte (per cent of R.B.C.)										Day of peak	Increase per cent	
	Before injection	Days after Injection											
		1	2	3	4	5	6	7	8	9			10
Injection of 240 mgm. in six doses													
543	1.3	1.3	1.0		1.0	2.9	1.9	4.0	7.2	3.6		3	453
544	1.8	1.2	0.5		2.5	1.2	2.0	3.4	2.8	1.7		7	126
545	0.6	1.5	2.0		1.6	0.5	1.3	2.8	2.1	1.3		7	150
546	0.7	1.2	0.7		2.2	1.6	4.0	6.1	3.4	2.3		7	512
547	1.1	1.0	2.2		1.1	0.6	1.3	3.5	3.7	1.8		8	146
548	0.2	1.1	1.0		0.8	2.0	2.0	1.7	3.0	2.0		8	500
Average	.95	1.36	1.23		1.53	1.46	2.08	3.58	3.53	2.43		7	211

quantity injected into each animal (240 mgm.) was divided into 6 doses. No toxic manifestations followed the administration of the dye. The peak of the reticulocytosis occurred on the 6th to 7th day, with a rise of 260 per cent over the initial value (Figure 1 and Table IV). There was no second reticulocytosis following an injection of 0.3 cc. of liver extract intramuscularly on the 15th day.

TABLE V

*Reticulocyte response in guinea pigs following intramuscular injection of normal gastric juice*

Guinea pig number	Injection	Reticulocyte (per cent of R.B.C.)												Day of peak	Increase	
		Before injection	Days after injection													
			1	2	3	4	5	6	7	8	9	10	11			12
1	4 cc. S.	2.1		1.7	1.7		1.8	4.3	4.0	6.4	7.4			3.4	9	252
2	4 cc. S.	2.5		2.5	2.9		3.6	4.7	9.8	4.6	3.6			2.4	7	292
3	4 cc. S.	2.1		2.4	3.9		2.6	6.2	7.4	4.0	3.8			1.4	7	252
3	0.2 cc. B.	1.6		3.4		1.6	5.1	3.1	1.0	2.8	4.8		0.8		5	
4	1 cc. S.	1.5		1.8	1.2		1.1	4.9	6.0	2.3	3.8			1.4	7	300
5	1 cc. S.	3.4		3.6	3.3		5.8	8.4	8.6	12.4	10.8				8	265
531	0.05 cc. Mo.	1.7			3.4	3.0	2.3	1.8	4.0	5.4	1.2				8	218
533	0.01 cc. Mo.	0.9			3.1	2.8	4.6	4.0	6.0	8.0	5.2				8	230
537	0.025 cc. Mo.	1.8			2.5	3.1	2.0	4.2	5.1	4.6	4.4				7	183
540	0.1 cc. no. 2	1.0		0.9			1.8	2.9	5.2	4.6	1.4			1.5	7	420
542	0.5 cc. no. 2	1.2		1.3			1.6	3.1	4.2	7.2	2.7			1.8	8	500
550	0.05 cc. Mo.	0.4			1.9	3.6	8.0	3.8	1.7	2.1					5	1900
550	0.3 cc. Ze.	0.9	1.1			1.8	3.2	7.1	4.2		2.3				6	688
551	0.1 cc. Mo.	0.6			1.3	1.2	1.6	3.3	1.2	1.3					6	450
552		0.7			1.8	1.7	3.5	2.0	2.1	2.5	1.0				5	400
553	0.025 cc. Mo.	2.4			4.2	3.7	2.3	7.9	3.2	2.4					6	210
554	0.05 cc. Mo.	1.4			2.0	2.4	3.6	4.8	1.0	1.5					6	240
557	0.5 cc. Sb.	2.0	2.5			0.8	2.1	6.4	3.2		1.0				6	229
573	0.02 cc. Sm.	1.2			0.4	0.6	1.1	3.8	2.6						6	216
574	0.25 cc. O.	1.6			1.5	1.7	2.9	4.1	4.0						6	156
Average		1.65			2.02	2.34	2.15	3.03	4.54	4.22	4.50	3.81		1.92		174

TABLE VI

*Reticulocyte response in guinea pigs following intraperitoneal injection of normal gastric juice*

Guinea pig num- ber	Injection	Reticulocyte ( <i>per cent of R.B.C.</i> )												Day of peak	In- crease
		Before injection	Days after injection												
			1	2	3	4	5	6	7	8	9	10	11		
549	0.5 cc. Bo.	1.2	1.1		0.6	1.3	0.5		1.3	1.8	2.7		3.9	11	<i>per cent</i>
550	0.5 cc. Bo.	2.2	1.4		1.6	2.0	1.4		1.0	0.6	0.9		1.1		225
554	0.5 cc. no. 2	0.5	1.1		0.5	1.3	1.0		1.7	3.4	3.8		2.9	9	263
557	0.5 cc. no. 2	0.4	1.0		1.0	1.2	2.4		2.2	1.9	2.0		2.5	11	256
564	0.1 cc. Mo.	1.5		1.7	0.9	1.6	1.2	2.2	3.6	2.6	2.4		2.2	7	140
565	0.05 cc. Mo.	1.1		2.1	1.3	0.7	2.0	1.0	0.6	2.4	1.8		1.4	8	120
566	0.025 cc. Mo.	1.5		1.1	2.8	1.9	1.8	1.2	1.0	1.2	1.6		0.2	3	87
574	0.5 cc. Sm.	1.6			1.2	0.6	1.2	1.7	1.2			0.4		6	8
Average		1.25	1.15	1.63	1.23	1.32	1.43	1.52	1.57	1.64	2.17		2.02	9	73

#### 4. Gastric juice

Filtered, unneutralized gastric juice from normal individuals was injected intramuscularly and intraperitoneally into reacting animals, 2 months or more after a liver injection. Only one injection was given, varying in amount from 4 cc. to 0.01 cc. The larger doses of gastric juice were injected undiluted, but a dilution of 1:10 was made in the case of the smaller amounts in order

to be able to measure the quantities more accurately.

(a) *Intramuscular injections:* Following the intramuscular injection the reticulocytes of the guinea pigs remained at the preinjection level until about the third or fourth day, after which they began to rise, reaching their peak from the 5th to the 8th day following the injection (Figure 2 and Table V). The average increase

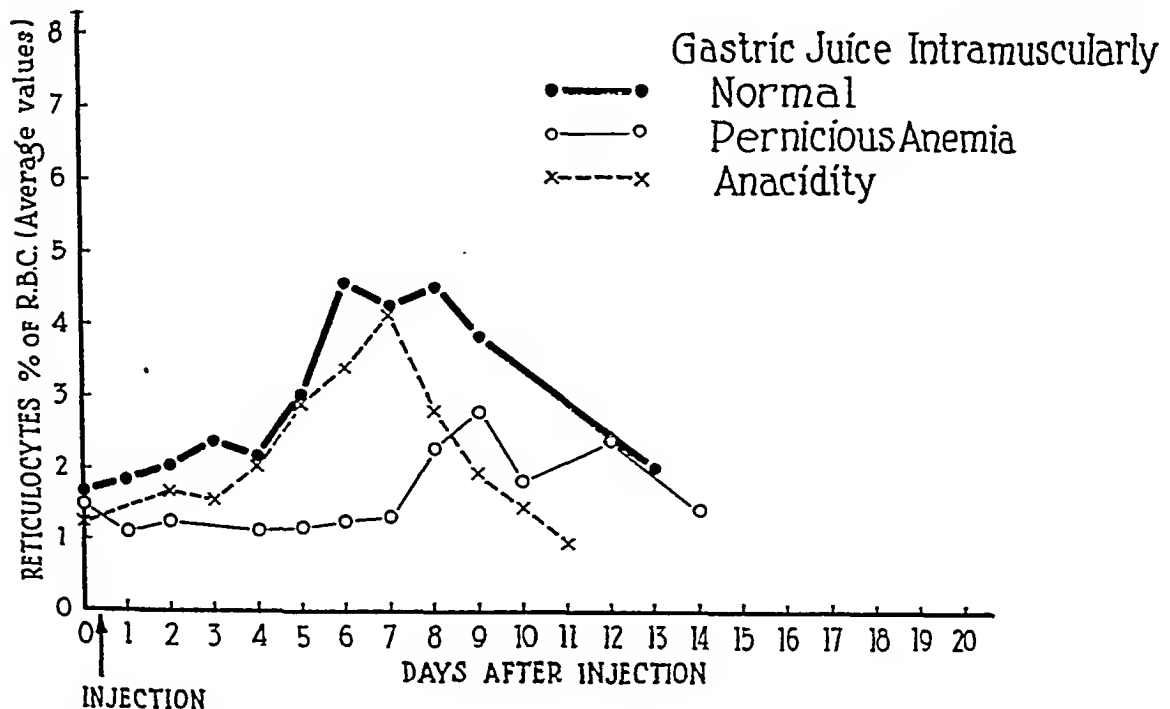


FIG. 2. RETICULOCYTE RESPONSE OF GUINEA PIGS TO INTRAMUSCULAR INJECTIONS OF GASTRIC JUICE

TABLE VII

*Reticulocyte response in guinea pigs following intramuscular injection of anacidity gastric juice*

Guinea pig num- ber	Injection	Reticulocyte ( <i>per cent of R.B.C.</i> )												Day of peak	In- crease
		Before injec- tion	Days after injection												
			1	2	3	4	5	6	7	8	9	10	11		
538	0.02 cc. E.	1.2	3.5		4.0	3.0	3.7	2.7	1.4	1.2		1.2	4	235	
536	0.5 cc. Me.	0.2		0.9	3.4	6.3	3.7	9.4	9.4	5.0			7 to 8	3700	
541	0.02 cc. E.	1.9	2.0		1.2	3.7	4.3	4.3	1.2	0.6		0.8	6 to 7	125	
544	0.1 cc. Ho.	1.0		0.6		2.7	2.2	2.7	2.4	1.5			7	170	
548	0.01 cc. E.	1.5	1.8		1.9	3.3	3.6	2.2	1.4			0.4	6	140	
555	1 cc. Hu.	0.9	0.6	0.9	0.3	0.5	1.8	3.2		0.3	1.0	0.7	7	255	
556	0.5 cc. Hu.	1.0	2.1	2.8	2.8	6.0	5.0	6.7		5.4	2.2	0.9	7	570	
557	0.1 cc. Hu.	1.1	0.6	0.5	1.0	1.2	2.7	5.0		2.4	1.0	1.1	7	355	
558	1 cc. L.	1.2	0.5	1.4		3.0	4.0	3.7	2.0		2.6		6	230	
560	0.1 cc. L.	2.4	2.2	3.2		1.8	4.9	6.0	1.6		0.3		7	150	
561	0.5 cc. E.	0.5	1.3	1.0	1.8	1.2	1.8	2.0	0.8	0.4			7	300	
562	0.1 cc. E.	1.1		1.8	1.7	2.5	2.8	3.2	4.6	1.4		1.8	8	320	
563	0.02 cc. E.	1.8		2.2	2.4	3.1	3.8	2.6	3.2	1.0		0.8	6	115	
Average		1.21	1.62	1.53	2.05	2.94	3.40	4.13	2.78	1.92	1.42	.96	7	240	

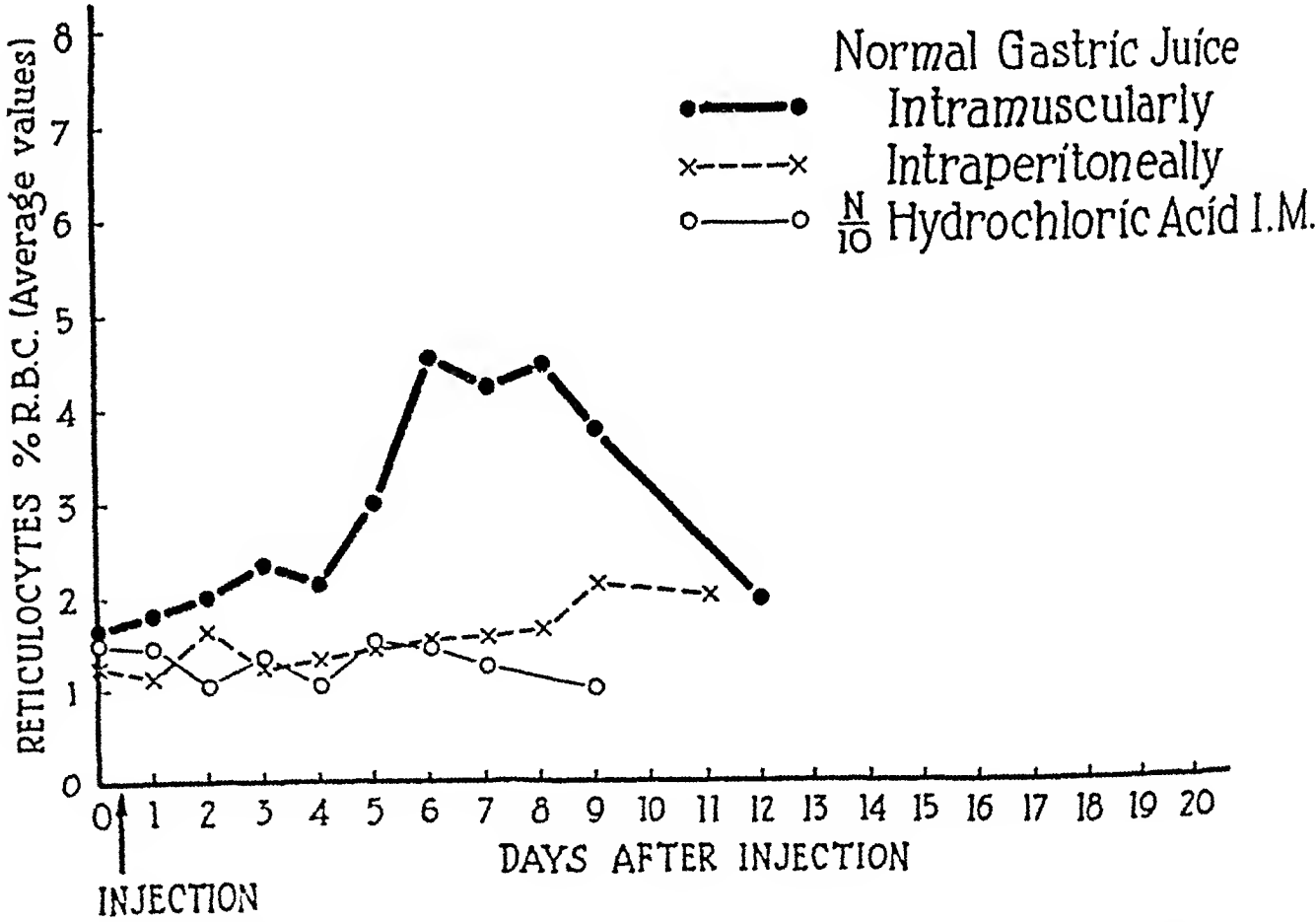


FIG. 3. RETICULOCYTE RESPONSE OF GUINEA PIGS TO INTRAMUSCULAR AND INTRAPERITONEAL INJECTIONS OF GASTRIC JUICE; AND INTRAMUSCULAR INJECTION OF HYDROCHLORIC ACID

TABLE VIII

*Reticulocyte response in guinea pigs following intramuscular injection of gastric juice from pernicious anemia patients*

Guinea pig number	Injection	Reticulocyte (per cent of R.B.C.)											Day of peak	Increase	
		Before injection	Days after injection												
			1	2	3	4	5	6	7	8	9	10			
538	0.2 cc. Mc.	1.6		2.4		3.0	3.4	1.4	2.4	2.2	2.6	1.8	5	per cent 113	
540	1 cc. K.	0.8		0.6		0.7	0.3	0.5	1.1	1.2			8	50	
541	.4 cc. Mc.	2.4		3.2		2.4	2.4	2.9	2.0	3.6	2.6		8	33	
543	0.1 cc. Hs.	1.1	1.8	1.7		1.4	1.2	1.5	0.8				2	54	
548	0.2 cc. Mc.	3.0		2.0		1.0	1.1	1.6	1.6	2.0	3.2		9	6	
548	0.5 cc. M.F.	0.7	1.0	0.4		0.9		1.0		1.0		6 to 8	17		
549	0.5 cc. H.	1.3	0.7	0.6		0.5	0.9	1.1	0.8				0		
550	0.1 cc. H.	2.9	2.3	1.6		0.5	0.6	0.2	1.1				0		
551	1 cc. H.	1.1	1.1	0.7		0.5	0.9	1.2	1.3				7	18	
552	0.5 cc. P.	1.7	0.8	0.7		1.1	0.7	0.7	0.7				0		
553	1.0 cc. P.	0.5	0.3	0.5		0.6	0.6	1.3	1.3				6 to 7	225	
554	0.2 cc. P.	0.3	0.7	0.2		0.3	0.3	1.2	1.2				6 to 7	140	
Average		1.45	1.08	1.21		1.07	1.13	1.21	1.30	2.25	2.8	1.8	8	55	

was 175 per cent. The per cent of rise of reticulocytes did not increase with the larger doses, and was approximately the same whether 4 cc. or 0.02 cc. of gastric juice were injected. The curve resembled closely that obtained following the intramuscular injection of liver extract. The injection of liver extract intramuscularly did not cause any further increase of reticulocytes.

(b) *Intraperitoneal injections.* Normal gastric juice, giving good responses when injected intramuscularly in doses as low as 0.02 cc., caused only slight and rather late (7th to 11th day) rise in reticulocytes (Table IX). This increase in reticulocytes was so irregular that the average curve was totally unlike that obtained with intramuscular injections, and showed a peak on the 9th day, with a percentage increase of only 73 (individual limits 8 to 256 per cent).

(c) *Anacidity gastric juice.* Gastric juice from patients having a constant gastric anacidity, i.e. secreting gastric juice following histamine stimulation with a pH less than 3 as shown by Töpfer's reagent (dimethyl-amino-azo-benzene) and no demonstrable blood or gastro-intestinal disease, was tested similarly on guinea pigs. The gastric juice was all injected intramuscularly, and the rise in reticulocytes resembled closely that obtained following the injection of normal gastric juice (Figure 2 and Table VI, average increase 240 per cent, peak on the 7th day, individual in-

creases 125 per cent to 4,600 per cent). The amounts injected ranged from 1 cc. to 0.02 cc. filtered gastric juice. Not all the animals reacted when 0.01 cc. of gastric juice was injected.

(d) The intramuscular injection of 0.5 to 0.2 cc. N/10 HCl caused no reticulocytosis whatsoever (Figure 3, Table VIII).

(e) *Pernicious anemia gastric juice.* Gastric juice was obtained from patients who had typical Addisonian anemia. They had been followed for varying lengths of time in the out-patient clinic, and were found to respond typically to the administration of liver extract. These patients were all at the beginning of a relapse and, with the exception of one, had had no treatment during the preceding three months. This one had received 3 cc. of liver extract (equivalent to 300 grams whole liver) intramuscularly the day before the juice was obtained.

Amounts varying from 1 cc. to 0.2 cc. of filtered gastric juice were injected intramuscularly into reacting guinea pigs. Except in one case, there was no reticulocytosis in any case for the first 7 days following the injection, but in 4 out of 12 cases there was a slight rise in reticulocytes on the 11th and 12th days, to drop again at or below the previous level on the 14th day (Figure 2). In this one exception there was a slight rise on the 5th day, to be followed by a drop, then a greater rise on the 12th day (Table VII). The

TABLE IX

*Reticulocyte response in guinea pigs following intramuscular injection of N/10 hydrochloric acid*

Guinea pig number	Injection	Reticulocyte (per cent of R.B.C.)										Day of peak	Increase per cent
		Before injection	Days after injection										
			1	2	3	4	5	6	7	8	9		10
544	0.2 cc.	1.4	1.3	0.8	0.8	0.6	1.8	0.9	1.5	1.0		5	12
547	0.2 cc.	1.6	1.8	1.2	1.7	1.6	1.4	2.4	1.5	1.0		6	50
548	0.5 cc.	1.5	1.2	1.2	1.5	0.9	1.3	1.0	0.7	1.0			
Average		1.5	1.43	1.06	1.33	1.03	1.5	1.43	1.23	1.0			

two other guinea pigs, injected with the same material, gave a response no greater than would be found in an uninjected animal. All the guinea pigs, however, responded promptly to intramuscular injections of 0.1 to 0.25 cc. of normal gastric juice administered on the 11th to 14th day with a reticulocyte peak on the 6th to 7th following this second treatment.

TABLE X

*Summary of experimental data*

Number of animals	Material used	Amount	Route*	Average reticulocytes at beginning	Average reticulocytes at peak	Average percentage increase	Average day of peak	Number of patients
5				0.85	1.36	90		
4	Liebig's beef extract	0.5 grams	I.m.	0.65	1.2	90		
28	Anti-pernicious anemia liver extract	0.1 cc. (3.3 grams liver)	I.m.	0.91	5.06	440	6	
6	Congo red	225 mgm. in 6 doses	I.p.	1.15	3.58	211	7	
20	Normal gastric juice	4 cc. to 0.01 cc.	I.m.	1.65	4.54	175	6	8
8	Normal gastric juice	0.5 cc. to 0.1 cc.	I.p.	1.2	2.02	73	11	4
3	N/10 HCl	0.5 cc. to 0.2 cc.	I.m.	1.3	1.4	7.5		
13	Anacidity gastric juice	1.0 cc. to 0.01 cc.	I.m.	1.21	4.13	210	7	6
12	Pernicious anemia gastric juice	1.0 cc. to 0.25 cc.	I.m.	1.67	2.8	170	9	6

\* I.m. = Intramuscular.

I.p. = Intraperitoneal.

Guinea pigs which had received injections of normal gastric juice and anacidity gastric juice did not respond further to an injection of liver extract or normal gastric juice on the 12th to 16th day, thus differing from the animals who received gastric juice from patients with pernicious anemia. The slight rise on the 12th day occurring in some of the latter animals is evidently due to a different mechanism, and is not a reaction in the sense given here.

#### DISCUSSION AND CONCLUSIONS

Several investigators have observed that guinea pigs respond by a reticulocytosis to injection (intramuscular, intraperitoneal or subcutaneous) of liver extract potent in the treatment of Addisonian anemia. We have obtained similar findings and have used this means of separating guinea pigs into two groups—reactors and non-reactors, the latter being discarded.

The same animals (reactors) were found to give a reticulocyte response following intraperitoneal injections of 1.5 per cent Congo red in 6 per cent glucose. The reaction was not quite so marked as that obtained with intramuscular liver extract, but it was similar in type and the animals had no further reticulocytosis following an injection of liver extract administered on the 16th day after the beginning of the Congo red treatment. Massa and Zolezzi (5) have recently confirmed this reaction in guinea pigs. Congo red further resembles liver extract in that it can be used to produce a remission in cases of pernicious anemia (6) (7). Others have failed to confirm this (23).

Castle and his coworkers (8) (9) showed that normal human gastric juice, incubated with beef muscle or with alcoholic extract of yeast, was able to produce a remission in patients suffering from pernicious anemia. The same substances have recently been shown to be reticulocytogenic for guinea pigs following oral administration (2) (4). Neither group of workers (4) (8) was able to obtain results with gastric juice alone. However, very recently Greenspon (10) produced a reticulocytosis in a pernicious anemia patient by feeding him neutralized and chilled normal gastric juice. The author felt that the neutralization and chilling inactivated the pepsin, which inactivated the anti-anemic factor present in gastric juice.

Furthermore, Morris and coworkers (11) and Fouts, Helmer and Zervas (12) have shown that intramuscular injections of large amounts of normal gastric juice, concentrated, caused a remission in pernicious anemia patients.

Singer (13) reported that rats respond to a single intramuscular injection of 1 to 20 cc. of normal, unheated gastric juice with an increase in reticulocytes, but that no such reticulocytosis was obtained with gastric juice from pernicious anemia patients. Fleischhacker and Schlesinger (14) also working with rats obtained a similar increase in reticulocytes following the injection of hydrochloric acid and pepsin, normal gastric juice or gastric juice from 50 per cent of the cases of pernicious anemia.

Salah (15), using Singer's method, induced a reticulocytosis in rats by giving injections of gastric juice from pellagrous patients. The advisability of using rats as test animals, however, has been questioned by Engberding (16) who showed that rats have a reticulocytosis following the mere taking of blood necessary for the counts.

The intramuscular injection of gastric juice in guinea pigs gave results similar to those reported by Singer with rats (13), but there was no response to tenth normal hydrochloric acid. The normal gastric juice and that from patients having an anacidity caused a reticulocytosis, while that from pernicious anemia patients gave no immediate response. The response from the gastric juice from pernicious anemia patients occurred only in a fourth of the cases, and at a later time, 11th to 12th day, than that found with normal gastric juice, and was not classed, therefore, as a true response. The minimal amounts necessary for evoking a response were practically the same with anacidity as with normal gastric juice, but the height of the reticulocytosis was slightly lower, indicating that the concentration of the reticulocytogenic agent was slightly less in anacidity than in normal gastric juice. None of the patients with gastric anacidity showed any anemia. Hartfall and Witts (17) testing the content of Castle's ferment in patients with simple achlorhydric anemia found that the amount present was less than in normal gastric juice, and quite variable in different patients.

The greater response with normal gastric juice

cannot be attributed solely to the presence of the acid, since the intramuscular injections of fairly large doses of tenth normal hydrochloric acid caused no reticulocytosis whatsoever.

There is no adequate explanation for the difference in action following intraperitoneal and intramuscular injection of gastric juice. The inability of normal gastric juice to be hematopoietically active when administered orally was attributed by Castle and his coworkers to the absence of an extrinsic factor to react with the intrinsic factor present in the gastric juice. The extrinsic factor was thought to be present in muscle and alcoholic extract of yeast, used in the incubation. Greenspon (10) feels that this lack of activity is due to antagonistic action of the pepsin towards the antianemic principle in the gastric juice. The pepsin is removed by absorption on muscle, or rendered inactive by chilling and neutralizing the gastric juice. Following this treatment, normal gastric juice has been stated to be hematopoietically active when administered orally, though it may have reacted with food eaten four hours later.

The results of Fouts et al. (12), on the other hand, fail to show any injury to the antianemic principle due to concentrating whole gastric juice in vacuo at 40° C., the temperature at which pepsin is most active.

The gastric juice was passed through an ultrafilter which held back both the pepsin and rennin. When the gastric juice was merely preserved in the ice box for two months before filtering, the concentrate alone had antianemic properties, while the filtrate was totally inactive. This showed that untreated active principle was not able to pass through the filter.

When, however, the material had been concentrated in vacuo at 40° C. both the filtrate and concentrate had antianemic properties, indicating either a physical or chemical change in the antianemic substance. That such a change must have taken place is further demonstrated by the fact that with fresh gastric juice neither the filtrate nor the concentrate had any hematopoietic activity.

Braun (18) suggested that gastric juice is not effective by mouth because the active principle does not pass ultrafilters and therefore cannot be absorbed from the gastro-intestinal mucosa. The

digestion with beef muscle, yeast extract or concentration in vacuo at 40° C. presumably causes a reaction between this active principle and a protein molecule, which then acts as a conveyor of the active principle through the mucous membranes. Braun (18) cites as corroborative evidence the fact that Congo red does not pass ultrafilters until attached to a protein molecule (19). Since we found that Congo red was hematopoietic when injected intraperitoneally, it is probable that the irregular reticulocytosis following intraperitoneal injections of active juice was not due solely to lack of absorption. One-fiftieth the amount of gastric juice injected intraperitoneally causes a reticulocytosis following intramuscular injection.

Since the same response was obtained following intramuscular injections of normal and anacidity gastric juice and with Congo red intraperitoneally, it is not possible to prove with certainty that the reticulocytogenic substance of gastric juice is identical with Castle's ferment.

Gastric juice from patients with Addisonian anemia contains less than one-fiftieth the amount of reticulocytogenic substance present in normal gastric juice.

Barnett (20), Castle et al. (21) and Beebe and Wintrobe (22) have used the demonstration of the presence or lack of Castle's "intrinsic" factor as a diagnostic test in cases of obscure anemia by incubating with beef muscle the gastric juice from the patient to be investigated and administering the mixture to a case of pernicious anemia. This test, however, took large amounts of gastric juice, and the presence of a known case of pernicious anemia in relapse. The results of injecting gastric juice intramuscularly into guinea pigs are just as striking as the administering of this gastric juice-beef muscle preparation to the patient, and more easily obtainable. We suggest that this reaction of the guinea pig be used as a diagnostic procedure in cases of obscure anemia. The amount of gastric juice necessary is very small, 1 to 2 cc. being ample. It is essential, however, to have the gastric juice free from bile; as otherwise large areas of necrosis are caused with amounts as small as 0.2 cc., and frequently death on the third or fourth day with larger amounts.

## SUMMARY

The presence of a substance in antipernicious anemia liver extract, reticulocytogenic for the majority of guinea pigs, was confirmed. A similar substance was demonstrated following intraperitoneal injection of Congo red and intramuscular injection of gastric juice from normal individuals, and also from patients having gastric anacidity but no anemia.

Irregular and late reticulocytosis was obtained following intraperitoneal injection of normal gastric juice. The mechanism of this altered activity is not clear.

The intramuscular injection of gastric juice from patients suffering from typical Addisonian anemia caused no reticulocytosis within the first 8 days. This reaction of the guinea pig to the intramuscular injection of gastric juice is proposed as a test in the diagnosis of anemia of obscure origin.

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# THE COMPARISON OF UREA WITH UREA + AMMONIA CLEARANCES IN ACIDOTIC DOGS

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In 1921 Nash and Benedict (11) advanced the hypothesis that urinary ammonia is formed in the kidney from some precursor in the blood. This conclusion was based upon the observation that the concentration of ammonia was higher in the renal vein than in the renal artery, that the quantity of ammonia in arterial blood was too small to supply the ammonia found in the urine, and that the arterial ammonia concentration was unaffected by acidosis, alkalosis, and nephrectomy, which procedures modify the excretion of ammonia. This hypothesis has been supported by Loeb, Atchley and Benedict (8) and Rabinowitch (13). The nature of the precursor is, however, not established; Bliss (2) has suggested amide nitrogen of protein, Krebs (7) has suggested amino nitrogen, and Nash and Benedict (11), Keeton (6), Mann and Bollman (9) have suggested urea nitrogen. Steenbock, Nelson and Hart (18) and Keeton (6) have claimed that in acidosis a reciprocal relation exists between the excretion of urea and ammonia nitrogen, such that the increased excretion of the latter is compensated by a decreased excretion of the former, but McCollum and Hoagland (10) and Adolph (1) have asserted that the total nitrogen excretion increases to an extent equivalent to the increased ammonia excretion.

Van Slyke, Page, Hiller and Kirk (21), accepting the hypothesis that ammonia is formed in the kidney from urea, have recommended that the sum of urea + ammonia nitrogen in the urine be used in calculating the equivalent of the urea clearance in acidosis. This urea + ammonia clearance, they believe, has the same functional significance in acidotic individuals as has the urea clearance under normal conditions. In support of their hypothesis they present comparisons of the urea clearance in normal men on a normal diet with urea + ammonia clearances on a low protein diet with induced acidosis.

It is known that in both dog and man (17) the urea clearance is considerably less than the rate of glomerular filtration, the deficit in the urea clearance being commonly attributed to the reabsorption of urea. It would be unjustified to link this deficit in the urea clearance with the process of ammonia formation, since it exists at all times, whether ammonia is being excreted or not. Nevertheless, any decomposition of filtered urea would reduce the urea clearance to a further extent, and thus supplement the normal deficit. (Such decomposition might occur either in the lumen of the renal tubules, or in the tubule cells after the reabsorption of a fraction of the filtered urea from the tubular urine). In fact, if the conclusions of Van Slyke et al. (21) are correct—i.e., if the urea + ammonia clearance in acidosis is of the same magnitude and has the same functional significance as has the urea clearance in the normal—it follows that ammonia excretion does in fact create a further deficit in the urea clearance, and that urinary ammonia is derived directly or indirectly from urea that has been initially present in the glomerular filtrate. And by the same reasoning, if the implied reciprocal relation between the clearances were an exact one, the formation of ammonia from any other nitrogenous precursor would be ruled out of consideration. Van Slyke, Page, Hiller and Kirk do not comment on these and other physiological implications necessarily issuing from their conclusions. The problem seemed, therefore, of sufficient interest to justify further examination.

There are certain features, moreover, in the observations of these investigators that render their interpretation uncertain. It is known that all renal clearances in both man and dogs are affected by the protein content of the diet (3, 5, 12, 14, 15). It seemed not beyond possibility that on a protein-poor diet such as was used by Van Slyke and his coworkers in acidotics, all

TABLE I

*A comparison of the urea, the urea plus ammonia, and the creatinine clearances at low and high plasma urea concentrations in the acidotic dog*

Total concurrent time	Urine flow per minute	Plasma		Urinary excretion rate		Plasma CO <sub>2</sub> combining power	Clearance			Clearance ratio	
		Urea-N	Creatinine	Urea-N	NH <sub>3</sub> -N		Urea	Urea+NH <sub>3</sub>	Creatinine	Urea Creatinine	Urea+NH <sub>3</sub> Creatinine
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per min- ute	mgm. per min- ute		cc. per 100 cc.	cc.	cc.	cc.	
min- ute	cc.						cc.	cc.	cc.		
Experiment 13, Dog S. B.											
15	11.6	1.13	14.2	.76	1.53	28.5	66.9	202	132	.51	1.53
30	11.1	1.13	14.4	.68	1.29		60.5	175	125	.48	1.40
45	10.1	1.13	14.4	.66	1.33		58.7	176	119	.49	1.48
47 (60)	20 grams urea intravenously									(.49)	(1.47)
75	7.25	71.5	14.2	36.7	1.49		51.3	53.4	91.4	.56	.58
90	4.59	62.5	13.3	35.6	1.65		57.0	59.6	103	.55	.58
104	3.38	57.4	12.7	31.2	1.52		54.4	57.0	95.6	.57	.60
										(.56)	(.59)

clearances might be reduced without any specific depression of the urea clearance. In one individual (Table II) they attempt to rule out this possibility by including the creatinine clearance as a standard of reference, but the data presented show such marked variations within themselves that they cannot confidently be interpreted.

We have therefore examined the excretion of urea and ammonia in the dog under more controlled and exacting conditions. Female dogs were used and fed a maintenance, low-protein diet, consisting of cracker meal with butter, yeast, cod liver oil and salt. To induce acidosis the dogs were given 5 cc. of concentrated hydrochloric acid well diluted with water each day for several days, and in later experiments they were given 2 liters of water the day before an experiment to induce diuresis and thus reduce the blood urea concentration. Urea was determined by the gasometric method of Van Slyke (19), using 2 cc. of plasma where the plasma urea was low. After the administration of sodium bicarbonate it was necessary to add excess acid to both plasma and urine to insure removal of preformed carbon dioxide in the determination of urea. Creatinine was determined on diluted urine and tungstic acid

TABLE II

*A summary of comparisons of the urea, the urea plus ammonia, and the creatinine clearances at low and high plasma urea concentrations in acidotic dogs*

Experiment number	Dog number	Number of periods averaged	Average urinary flow	Average plasma urea-N	Plasma CO <sub>2</sub> combining power	Average NH <sub>3</sub> -N excreted	Clearance			Clearance ratios	
							Urea	Urea+NH <sub>3</sub>	Creatinine	Urea-Creatinine	Urea+NH <sub>3</sub> -Creatinine
			cc.	mgm. per 100 cc.	cc. per 100 cc.	mgm. per minute	cc.	cc.	cc.		
1	2	3	5.2	1.84		.79	60.9	104.1	104.7	.58	.99
		3	3.6	64.0		.91	44.2	45.6	79.3	.56	.57
2	2	3	3.4	2.19		.55	51.2	76.1	94.0	.54	.80
		3	4.6	74.8		.79	42.3	43.3	78.4	.54	.55
4	4	3	6.4	1.98	37	.92	45.0	91.3	83.8	.54	1.09
		3	3.4	78.4		1.07	36.6	38.0	63.5	.53	.60
5	4	3	6.4	2.50	36	.89	47.0	82.7	89.5	.53	.92
		3	3.8	73.4		.96	37.5	38.8	68.4	.55	.57
7	1	3	3.6	1.60	41	.49	24.1	54.6	46.8	.52	1.17
		3	4.9	111.0		.61	24.2	24.8	40.0	.60	.61
10	S.A.	3	7.5	1.40	30	1.01	50.8	122.0	115.0	.44	1.06
		3	3.4	60.3		.99	40.4	42.0	81.1	.50	.52
11	S.A.	3	0.9	3.25	35	.96	48.3	77.7	103.0	.47	.75
		3	5.1	65.0		1.33	37.4	39.4	76.5	.40	.51
13	S.B.	3	10.9	1.13	28.5	1.38	62.0	184.0	125.0	.50	1.47
		3	5.1	63.8		1.55	54.2	56.7	96.5	.56	.59

filtrates of plasma according to Shannon, Jolliffe and Smith (16).

Ammonia determinations in Experiments 1 to 7 were performed by the permittit-Nesslerization method of Folin and Bell (4) and in Experiments 8 to 12, by the aeration-titration technique of Van Slyke and Cullen (20).

On the basis of the observations of Shannon (14), the creatinine clearance was used as a measure of the rate of glomerular filtration. Simultaneous creatinine and urea clearances were compared to eliminate errors arising from variations in glomerular activity and in order that the urea/creatinine clearance ratio would be available to indicate any change in the excretory mechanism of urea, independent of a change in glomerular activity.

Data on a typical experiment in which urea was administered intravenously during extreme acidosis are given in Table I. When the blood urea is low and ammonia furnishes a large fraction of the nitrogen in the urine, both the urea clearance and the urea/creatinine clearance ratio are within the usual range previously observed in dogs on a low protein diet. The urea + ammonia clearance, on

the other hand, is three times the urea clearance, and considerably above the creatinine or glomerular clearance, an unprecedented situation, for never heretofore has the urea clearance been observed to rise to the level of, much less exceed, the creatinine clearance in any mammal. When the plasma urea is high, and urea predominates over ammonia in the urine, the urea + ammonia clearance approximates the urea clearance observed at the low urea levels, since under these conditions no great elevation in clearance is introduced by the inclusion of ammonia in the calculation. The necessity for a standard of reference (creatinine clearance) for glomerular activity is evident in this experiment, since both the urea and the creatinine clearances fell in consequence of the intravenous injection.

The results obtained in eight experiments of the above type on five dogs are summarized in Table II. In each case three values obtained at low plasma urea levels and three values obtained at high plasma urea levels are averaged. These experiments are concordant in leading to the conclusions reached above, namely, that the inclusion of ammonia in the clearance calculation leads to an erroneously high result, the discrepancy depending on the degree of acidosis and the height of the blood urea. On the other hand, the true urea clearance is essentially the same, relative to creatinine, at all plasma levels of urea. It is evident that the excretion of urea and ammonia are in no wise reciprocally related, and if ammonia is derived from urea it must come at least in part from a source other than urea filtered at the glomerulus.

A second type of experiment was performed in which, after a series of control periods in acidosis, sodium bicarbonate was administered to render the urine alkaline. It was not possible to obtain an immediate reduction of ammonia excretion in all cases, three hours or more being required to effect this in some instances, even after large doses of alkali intravenously and by mouth. A typical experiment for the determination of the effect of sodium bicarbonate is given in Table III. The administration of alkali, by reducing the excretion of ammonia, reduced the urea + ammonia clearance markedly, relative to the creatinine clearance (the reduction was still in prog-

TABLE III

*A comparison of the urea, the urea plus ammonia, and the creatinine clearances in the dog under conditions of acidosis and alkalosis*

Total concurrent time	Urine flow per minute	Plasma		Urinary excretion rate		Plasma CO <sub>2</sub> combining power	Clearance			Clearance ratio	
		Urea-N	Creatinine	Urea-N	NH <sub>3</sub> -N		Urea	Urea+NH <sub>3</sub>	Creatinine	Urea	Creatinine
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per minute	mgm. per minute	cc. per 100 cc.	cc.	cc.	cc.	cc.	cc.
minutes	cc.										
Experiment 12, Dog S.A.											
15	9.47	1.40	18.8	.72	1.16	33	51.4	135	105	.48	1.25
30	7.57	1.40	17.5	.69	1.09		49.5	128	111	.45	1.15
45	4.60	1.40	16.5	.58	.95		41.5	110	95	.43	1.15
50											
Sodium bicarbonate administered											
(300)											
315	7.47	1.60	14.5	1.09	.52	89	63.2	101	122	.56	.83
330	6.00	1.60	15.2	.99	.25		61.9	77.2	113	.55	.83
345	4.73	1.60	15.4	.97	.11		60.6	67.4	113	.54	.80

ress at the conclusion of the experiment), whereas the urea/creatinine clearance ratio was affected to only a small degree.

Table IV is a summary of 4 such experiments on 4 dogs. It is evident from these experiments that the urea/creatinine clearance ratio is essentially the same in acidosis as in alkalosis. These experiments may also be taken, therefore, to indicate that the inclusion of ammonia in the urea + ammonia clearance of acidosis introduces an error, the magnitude of which is determined by

TABLE IV

*A summary of comparisons of the urea, the urea plus ammonia, and the creatinine clearances in the dog under conditions of acidosis and alkalosis*

Experiment number	Dog number	Number of periods averaged	Average urinary flow	Average plasma urea-N	Plasma CO <sub>2</sub> combining power	Average NH <sub>3</sub> -N excreted	Clearance			Clearance ratios	
							Urea	Urea+NH <sub>3</sub>	Creatinine	Urea	Creatinine
			cc.	mgm. per 100 cc.	cc.	mgm. per minute	cc.	cc.	cc.	cc.	cc.
3	2	3	2.5	2.16	17.5	.71	50.5	82.2	65.8	.77	.83
		3	6.6	2.61	75	.68	55.4	61.0	105.3	.53	.53
6	4	3	5.5	1.55	31	1.63	29.3	104.3	72.2	.50	1.31
		3	5.3	1.40	85	.50	42.2	85.0	82.1	.50	1.04
12	S.A.	3	7.4	1.40	33	1.67	47.5	123.9	104.9	.45	1.18
		3	6.1	1.60	89	.53	63.8	61.8	112.2	.53	.45
14	S.B.	3	10.3	.52	35	1.23	12.5	277.0	112.0	.44	2.84
		3	4.6	1.77	92	.19	62.9	73.0	125.8	.50	.51

the relative fraction of ammonia in the urine. From both groups of experiments (Table II and IV) we would conclude that the significance of the urea clearance in acidosis, alkalosis, and in the normal animal is the same.

The question may be raised whether observations on dogs are pertinent to man. The work of Steenbock et al. (18) on calves, of Keeton (6), and of Nash and Benedict (11) on dogs, has been applied to man as the basis of the paper of Van Slyke, Page, Hiller and Kirk (21). We entirely concur in this procedure; it appears at the present time that the urea clearance in man and dog has the same physiological significance, and is similarly related to the rate of glomerular filtration, showing a deficit below the latter in both species for reasons as yet unexplained. The excretion of ammonia in both species has likewise the same general physiological significance, so far as our present information goes. It therefore appears justified to continue to apply results obtained on dogs to man until carefully controlled experiments indicate otherwise.

One of the reasons why Van Slyke et al. (21) recommend the inclusion of ammonia in the determination of the urea clearance in man is the technical ease of the hypobromite method, which includes both forms of nitrogen. Inasmuch as these investigators and others have shown that in the normal and in the nephritic, urinary ammonia nitrogen is never more than a small fraction of the total nitrogen, this method of analysis would not introduce a significant error. But in diabetic acidosis or other conditions where a significant proportion of the urinary nitrogen might be in the form of ammonia, the clearances so obtained would be excessively high, and might even exceed the rate of glomerular filtration as indicated by the inulin clearance (17), even as the urea + ammonia clearances observed here in the dog exceed the creatinine clearance.

Our present experiments throw no light on the nature of the precursor from which urinary ammonia is formed. If urea is that precursor, it is evident that it is not the urea that has passed into the glomerular filtrate. It is, of course, possible that urea might be removed directly from the post-glomerular blood and transformed and deposited as ammonia in the tubular urine by the activity of the tubule cells. But it is just as

harmonious with the above presented experimental facts to suppose that urinary ammonia may be formed from amide nitrogen, as suggested by Bliss (2) or from amino nitrogen, as suggested by Krebs (7), or from some other precursor, through the agency of tubule cells.

### CONCLUSIONS

The urea clearance in the dog relative to the creatinine (glomerular) clearance is essentially the same in acidosis and alkalosis as in the normal.

At low plasma urea values in acidosis the urea + ammonia clearance is considerably higher than the urea clearance and may exceed the creatinine (glomerular) clearance. The magnitude of this discrepancy between the urea and the urea + ammonia clearances depends on the degree of acidosis and the height of the plasma urea, being greater the more marked the acidosis and the lower the plasma urea.

The urea + ammonia clearance in acidosis does not have the same significance nor is it of the same magnitude as the urea clearance in the normal animal.

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# RESPONSE TO INSULIN AS AN INDEX TO THE DIETARY MANAGEMENT OF DIABETES

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Recently there has developed a widespread trend toward allowing relatively large amounts of carbohydrate in the diabetic diet. It should be emphasized, however, that the use of higher carbohydrate diets is not new; Donkin's milk treatment (1874) may have provided 250 grams of carbohydrate daily, and the "cures" of von Düring, Mosse, von Noorden and others match in carbohydrate content many of those advised today (1). Despite the good results obtained in some cases with such diets, the treatment of diabetes was in general carried out upon principles established by Naunyn (2), who found it usually necessary to limit carbohydrate to very small amounts. It was still the common opinion of students of the disease that deleterious results followed the excessive consumption of carbohydrate, with loss of sugar tolerance and deterioration in the clinical condition of the patient.

Strict limitation of the carbohydrate in the diet has in general proved quite satisfactory treatment, and its use is based upon long clinical experience and is supported by excellent laboratory evidence. The lowest carbohydrate diets, introduced by F. M. Allen et al. (3), were based upon the observation that partially depancreatized dogs lost tolerance with high carbohydrate intake, but gained tolerance with low carbohydrate. Patients under Allen's observation seemed to act similarly. The low caloric, low carbohydrate diets of the "Allen era" were followed for years quite generally and with considerable success. It was the common opinion that excessive carbohydrate ingestion might exhaust the diabetic's pancreas.

More recent efforts to find the optimum diabetic diet have led in widely divergent directions. Newburgh and Marsh (4) and Petrén (5) found that high fat was well tolerated by many patients, and advised keeping the carbohydrate very low. Rosenberg (6) reports good results with a similar regime. The introduction of insulin permitted the use of higher carbohydrate

and encouraged many at least temporarily to forget the principle of "sparing" the carbohydrate metabolism. The leaders in the recent movement toward higher carbohydrate diets (Sansum; Adlersberg and Porges; Geyelin; and Rabinowitch (7)) have demonstrated that many diabetics experience a definite improvement in health and may gain in sugar tolerance upon diets higher in carbohydrate than previously thought advisable. This has been a significant advance in our knowledge, reemphasizing the observations of earlier workers and tending to modify previously accepted conceptions of the pathological physiology of the disease. It has been observed that many patients do not gain tolerance with high carbohydrate, but the fact that many do requires a revision of the idea that "pancreas-sparing" is necessary in the treatment of all patients with diabetes.

It is quite generally accepted that as concerns total calories, normal or slight undernutrition is advisable, and that 0.75 to 1.25 grams protein per kilogram per day should be given. Concerning the carbohydrate-fat ratio, however, on one side we find advocates of high fat to rest the pancreas, and on the other those recommending high carbohydrate to stimulate the pancreas. That this is true now, twelve years after the introduction of insulin, emphasizes that the use of the pancreatic hormone has so far not helped much in solving the problem. With so many theoretical, practical and experimental arguments on each side, it is not surprising to find the conservative, moderate fat, moderate carbohydrate regime as advocated by Joslin (8) so widely used, since it represents a compromise between the two extremes.

It seemed to us, however, an important observation that some of our diabetic patients gained tolerance with high carbohydrate, while others showed no tendency to gain, or even lost tolerance. It seemed possible that there might be some fundamental difference in the type of diabetes in pa-



tients who responded so differently. The widespread use of the conservative, compromise type of diet might conceal latent possibilities of great improvement in glucose tolerance, or might in some cases even cause loss of tolerance.

Accordingly an investigation was undertaken to determine whether diabetics who will gain tolerance on high carbohydrate can be clinically distinguished from those who will not. The primary object was to find, if possible, a method for deciding upon the optimum type of diet for each individual diabetic. The optimum diet should supply the necessary calories in palatable form with the lowest possible insulin dosage, and should permit the development of the greatest possible carbohydrate tolerance.

#### RELATIVE INSULIN RESPONSE

Three years ago studies were reported (9) confirming the observations of Falta and Boller (10) upon the frequency of relative insulin-resistance in diabetes. Since then we have found that the diabetics we have studied have tended to fall into two groups, the relatively insulin-sensitive, and the relatively insulin-resistant. The insulin-resistance here described is not that commonly indicated by the term, in which several hundred units per day are required. Infection, coma, hyperthyroidism, pituitary and adrenal disorders, hepatic and various cutaneous diseases account in most instances for temporary marked unresponsiveness to insulin. The resistant patients in this study showed a relative lack of response to insulin slight to moderate in degree and not explainable upon the basis of any discernible complications. The relative degree of insulin sensitivity tended to remain as a persistent characteristic of the individual.

Studies were planned to determine whether the two groups differed in their response to high carbohydrate diets. Fifteen patients were chosen, each of them being intensively studied for three weeks to three months in the hospital, and from three months to three or more years in the outpatient department. The only criterion employed in the selection of cases was the individual's willingness to cooperate. Observations have been made upon the response of these patients to high and low carbohydrate diets, and the influence of

these diets upon the concentration of blood sugar, the glycosuria, the ketonuria, the insulin requirement, the glucose tolerance and relative insulin sensitivity.

#### METHODS OF STUDY

The studies are best described under three headings: (1) determinations of relative insulin-sensitivity; (2) hospital studies; (3) observations in the outpatient department.

##### *1. Relative response to insulin*

(a) Blood sugar curves for four hours following the subcutaneous injection of one unit of insulin per 10 pounds body weight into the fasting patient were determined.

(b) Intravenous insulin tolerance was determined as a check upon the subcutaneous method. One-fifteenth unit per kilogram body weight was given intravenously to the fasting patient, blood samples for sugar analysis being collected every fifteen minutes for one and one-half hours.

(c) Tolerated overdose. After the insulin requirement upon the standard diet was determined, the total dose of insulin was gradually increased from day to day until the patient experienced a hypoglycemic reaction. The tolerated overdose may be defined as the amount of insulin above the insulin requirement which a patient will tolerate per day without having a reaction.

(d) Glucose equivalent. The glucose equivalent, representing the number of grams of glucose metabolized per unit of insulin, was calculated by dividing the number of grams excreted by the number of insulin units required to eliminate the glycosuria.

##### *Laboratory methods:*

(a) Blood sugar determinations were performed on capillary blood by the method of Somogyi (11). These are "true sugar" readings and average 15 to 20 mgm. per 100 cc. lower than those obtained by the older methods. Ante- and post-cibus blood sugars (fasting, and 2½ hours after breakfast) served as frequent checks upon changes in tolerance.

(b) The twenty-four hour urines were tested daily for glucose and acetone bodies. The quantitative excretion was determined by the Shaffer-Hartmann method (12).

(c) Glucose tolerance tests were performed by estimation of the fasting blood sugar, and at one-half, one, two and three hours after the ingestion of 0.8 gram dextrose per pound of body weight. For comparison with the figures of others the relative rise in the blood sugar is indicated by the percentage increase above the fasting figure. A more accurate measurement expresses the differences in area of the curves in "milligram-minutes" (Table IV). The figures given represent the rise above the fasting level in each case, a rise of 20 milligrams lasting for one hour, for example, being expressed as a rise of 1200 milligram-minutes.

(d) The total glucose value of the diets was calculated as 10 per cent of the fat plus 52 per cent of the protein plus 100 per cent of the carbohydrate.

Accurate dietary regulation and prompt collection and preservation of specimens for analysis was carried out by the trained staff of the Tirrill Metabolism Ward.

## 2. Studies on patients in the hospital

(a) Control diets were given supplying 27.3 calories per kilogram body weight. This was considered a maintenance energy value for patients spending most of their time in bed, but permitted to be up and about in the ward. One gram of protein, 1.7 grams of fat and 2 grams of carbohydrate per kilogram per day were chosen as representing an average between the high fat and high carbohydrate types of diet. The protein supplied 15 per cent, the fat 56 per cent and the carbohydrate 29 per cent of the total caloric value. In the high carbohydrate "normal type" diet used by Geyelin (7) fat furnishes 33 per cent of the calories or less, and the carbohydrate 53 per cent or over. Rabinowitch's high carbohydrate diets (7) supplied 22 per cent of the calories from fat, 63 per cent from carbohydrate. In the Newburgh high fat diets (4) fat formed 75 per cent, carbohydrate 10 per cent, of the caloric intake.

The daily glycosuria in grams was measured, and the daily amount of insulin necessary to keep the blood sugar within approximately normal limits, and the glycosuria below 10 grams per 24 hours was determined. No effort was made to get the urine absolutely sugar-free, since we should then have been unable to tell whether the actual daily insulin requirement was being exceeded.

After a period upon the control regimen sufficiently long to avoid marked variations in daily blood sugar or daily glycosuria, the effect of alterations in the diet was studied. These were arranged in such a way as to involve no change in the protein or total caloric intake or in the insulin dosage. Test diets of the high fat type and of the high carbohydrate type were given, and the effects upon glycosuria and glycemia noted.

In some of the cases studied, glucose tolerance and insulin tolerance tests, while on the control regime and after the high carbohydrate period, were performed.

In certain instances the addition of fat or carbohydrate to the control diet was employed to determine the effect of adding calories above the control level in the form of carbohydrate or fat.

(b) In some cases a second type of control diet was used supplying 60 grams protein, 125 grams fat, and 150 grams carbohydrate, 1965 calories, without regard to the patient's weight. The effect of added carbohydrate and fat could then be studied without regard to certain theoretical objections to the first method. In thin patients receiving only 1500 or 1600 calories on the first type of control diet, for example, changes in tolerance might be ascribed to undernutrition, while obese patients receiving 2700 calories would certainly be subject to possible tolerance changes from excessive food intake as well as to possible influences of the high carbohydrate or high fat diets.

## 3. Outpatient observations

The effects of varying the proportions of fat and carbohydrate in the diet of patients measuring and pre-

paring their own food at home and seen at intervals of a week or more could not of course be as closely judged as those in the hospital. Certain facts, however, make these observations of considerable importance, and permit placing them on a plane of equal significance with the hospital studies.

All of the outpatients had previously been in the hospital where the technique of weighing and measuring diets and of testing the urine for sugar had been well learned. Each outpatient had also been a subject of one of the hospital studies, so that hospital and outpatient studies serve as checks on each other. Frequent urine tests at home, at least once daily, were recorded, and the record brought with the patient on each visit. Frequent blood sugar determinations were made. On each visit an analysis of the patient's dietary control was made by the dietitian. Adherence to the prescribed total caloric intake could be checked by the frequent records of body weight.

With these considerations in mind, plus the fact that changes in carbohydrate tolerance observed over long periods of time should naturally be more significant in the study of diabetes, the results seem particularly significant.

## RESULTS

### 1. Relative response to insulin

(a) Subcutaneous insulin tolerance. The patients fell into two groups, the relatively insulin sensitive and the relatively insulin resistant (Table I). The sensitive group of eight patients exhibited a marked fall in the blood sugar, ranging

TABLE I  
Insulin tolerance (1.0 unit per 10 pounds subcutaneously)

Case number	Blood sugar (mgm. per 100 cc.)					Per cent fall
	Fasting	1 hour	2 hours	3 hours	4 hours	
INSULIN-SENSITIVE TYPE						
1	156	124	71	41(r)*	41	74
2	234	179	143	66	58	75
3	176	134	39(r)*	52	70	70
4	119	88	46	45	45	62
5	218	133	87	59	63	73
6	212	147	111	61	71	71
7	142	119	100	42	48	70
8	202	175	123	68	81	61
INSULIN-RESISTANT TYPE						
9	164	132	111	125	128	32
10	134	137	119	110	80	40
11	191	166	128	91	102	53
12	119	96	99	71	82	40
13	115	96	80	80	76	34
14	212	206	155	100	110	53
15	173	155	115	102	111	40

\* (r) indicates symptoms of hypoglycemia.

from 61 to 75 per cent of the fasting levels. All of them reached a blood sugar level of 68 or below, the average of the lowest determinations being 52 mgm. per cent. Definite signs of hypoglycemia were observed in every patient of this group and two of them experienced quite severe reactions.

The relatively resistant patients showed much less response. The per cent fall ranged from 32 to 53; none of them reached a point below 71 mgm. per 100 cc., and the average of the lowest determinations was 90 mgm. Signs of hypoglycemia were minimal or absent.

(b) Intravenous insulin tolerance tests upon three of the patients in each group indicated that possible differences in absorption of the subcutaneous insulin could not explain the observed differences in response. In each instance a patient sensitive to subcutaneous insulin was found also to be sensitive to intravenous insulin. Those relatively resistant showed relatively poor response to both the subcutaneous and the intravenous hormone (Table II). The relative fall in per cent ranged from 34 to 45 in the sensitive group, and from 12 to 22 in the resistant type.

TABLE II

*Insulin tolerance (1/15 unit per kilogram body weight intravenously)*

Case number	Blood sugar (mgm. per 100 cc.)							Per cent fall
	Fast-ing	15 min-utes	30 min-utes	45 min-utes	60 min-utes	75 min-utes	90 min-utes	
INSULIN-SENSITIVE TYPE								
1	213	200	166	159	141	145	160	34
3	156	127	110	96	86	94	98	45
8	189	170	156	145	130	112	135	40
INSULIN-RESISTANT TYPE								
11	182	177	175	174	170	168	158	13
14	206	202	199	181	182	192	194	12
15	194	190	173	157	163	151	160	22

(c) The tolerated overdose in those patients found to be sensitive in the insulin tolerance tests was in every case practically zero. An increase of as much as three or five units produced hypoglycemia, while a reduction of three or five units below the required amount led promptly to glycosuria and hyperglycemia (Table III).

TABLE III

*Clinical characteristics and insulin requirements*

Case number	Age	Sex	Nutrition	Blood pressure	Insulin requirement	Tolerated overdose	Glucose equivalent
	years			mm. Hg	units per 24 hours	units per 24 hours	
INSULIN-SENSITIVE							
1	31	M	Thin	104/70	30	0	1.3, 2.5, 2.1
2	46	M	Thin	106/74	75	0	1.9, 2.3
3	47	F	Slightly obese	138/74	0	0	3.2
4	29	M	Normal	110/70	50	0	1.1
5	38	F	Normal	105/70	50	0	1.8
6	44	F	Thin	105/70	38	0	2.9
7	53	F	Obese	174/88	0	0	
8	33	M	Thin	120/70	70	0	1.2
INSULIN-RESISTANT							
9	56	F	Normal	135/70	25	30	0.80, 0.90
10	68	M	Normal	140/80	60	75	0.22, 0.33
11	43	F	Very obese	190/120	50	60	0.37, 0.35, 0.40
12	53	M	Very obese	140/80	40	35	1.1
13	33	F	Obese	130/85	45	60	0.56, 0.50
14	53	F	Obese	135/70	55	65	0.70, 0.77
15	55	F	Obese	135/80	70	15	0.30

In the resistant group, however, increase in the insulin dosage had a relatively slight effect. In the seven patients in this group (Cases 9 through 15) the tolerated daily overdose ranged from 15 to 75 units. In Case 9 the requirement was 25 units, the tolerated overdose 30 units, so that 55 units daily were required to provoke hypoglycemia. In Case 10, the requirement was 60, the tolerated overdose 75, a total of 135 units causing the first evidences of insulin excess. In Cases 11, 13 and 14 the required dose could also be more than doubled without producing symptoms of hypoglycemia.

(d) The glucose equivalents, representing the number of grams of glucose metabolized per unit of insulin, would be expected to be higher in the insulin-sensitive group. Such was found to be the case, the values in this group ranging from 1.1 to 3.2 and averaging over 2 grams (Table III). In the resistant type the values averaged 0.55 gram, ranging from 0.22 to 1.1. On the average, then, by this criterion, there appears to be approximately four times as much effect per unit in the sensitive group as in the resistant group.

*Clinical characteristics*

While it is our impression that the clinical features in the two groups differ significantly (9), there is considerable overlapping of clinical characteristics (Table III). Those patients showing relative resistance are usually older, frequently are obese, and often have vascular hypertension. There is little tendency to acidosis, while the sensitive group develop acidosis and coma much more easily. The sensitive group are usually younger, are often thin, and have as a rule low blood pressures. It is doubtful, however, whether clinical features alone will serve to distinguish the two types. They cannot be distinguished as to severity, since the insulin requirement is on the average higher in the resistant group, but acidosis occurs more frequently in the sensitive type.

TABLE IV  
*Effect of high carbohydrate diets upon insulin tolerance and glucose tolerance*

Case number*	Diet (27.3 calories per kgm.)	Subcutaneous insulin tolerance	Intravenous insulin tolerance	Glucose tolerance	
				grams carbohydrate per kilogram	per cent fall
1 (S)	2 (6 days)	74	34	21,705	67
	3 (7 days)	70	32	29,145	125
4 (S)	2 (6 days)	62		23,220	251
	3 (9 days)	58		37,995	280
8 (S)	2 (11 days)	61	40	18,600	103
	3 (14 days)	60	38	23,170	104
11 (R)	2 (7 days)	53		13,560	92
	3 (6 days)	58		12,120	68
14 (R)	2 (16 days)	53	12	37,380	122
	3 (9 days)	60	28	25,410	82
15 (R)	2 (18 days)	40	22	24,270	96
	3 (8 days)	49	30	9,060	51

\* Cases 1, 4 and 8 (S) are relatively insulin-sensitive. Cases 11, 14 and 15 (R) are relatively resistant to insulin.

The clinical characteristics, with the insulin requirements, tolerated overdoses, and glucose equivalents are summarized in Table III. Cases 1 through 8 are those who showed relative sensitivity in the insulin tolerance tests. Cases 9 through 15 were relatively resistant.

*Results of dietary studies*

In Tables IV, V and VI analyses are given of the responses to the various test diets. Of eight relatively insulin-sensitive patients studied in the hospital, seven showed no gain or a loss of tolerance following high carbohydrate diets; one sensi-

tive patient gained tolerance. High fat, however, was relatively well borne and was followed in several instances by improved tolerance.

Seven relatively resistant patients studied in the hospital all showed definitely improved tolerance following high carbohydrate diets. Fat was as a rule poorly tolerated by this group and tended to impair tolerance.

In the outpatient studies over long periods of time five of six sensitive patients failed to gain tolerance with increased carbohydrate intake; one showed improved tolerance.

Five relatively resistant patients gained tolerance in a definite and remarkable manner upon progressively increasing the carbohydrate ingestion.

*The cause of improved carbohydrate tolerance resulting from high carbohydrate diets*

It is evident from these experiments that some patients with diabetes may be expected to gain tolerance when allowed large amounts of carbohydrate, while others either fail to gain or actually lose tolerance.

The early observation of Hamman and Hirschman (13) that the hyperglycemia resulting from the second of two equal ingested amounts of glucose by a normal subject is less than that following the first dose has been confirmed by Staub (14), Traugott (15), Foster (16), du Vigneaud and Karr (17), and Lennox (18). Lennox demonstrated that this was true whether the glucose be given orally or intravenously. This effect is now commonly referred to as the Staub-Traugott phenomenon.

Sweeney (19) investigating the effects of various diets upon normal subjects, and using the glucose tolerance test as an indicator, concluded that starvation or a high fat diet decreased the tolerance, protein diets had little effect, and high carbohydrate raised the tolerance. He explained this effect by supposing that the mechanism secreting insulin becomes increasingly sensitive to stimulation when frequently subjected to the higher blood sugar resulting from the high carbohydrate diets, while the absence of such stimulation caused insulin to be secreted less readily and in smaller amounts.

TABLE V  
Responses to various test diets

Case number	Age and sex	Diet number	Days on diet	Protein	Fat	Carbo- hydrate	Calories	Total glucose value	Insulin	Blood sugar (a.c./p.c.)	Glycosuria	Ketonuria	Remarks
	years			grams	grams	grams		grams	units per 12 hours	mgm. per 100 cc.	grams per 24 hours		
1	31 M	1	6	60	102	120	1640	165	30	100/93	2.1		Control period
		2	4	60	89	150	1640	194	30		29.2		High carbohydrate; quantitative excretion, 27.1 of 29 grams
		3	3	60	60	215	1640	256	30	/391	79.9	++	Higher carbohydrate; quantitative excretion, 50.7 of 62 grams
		4	4	60	102	120	1640	165	30	/99	6.8		Control diet, loss of tolerance after high carbohydrate; glycosuria higher than in control period
		5	4	60	138	40	1640	88	30(R) 10	/90	0		High fat, insulin decreased
		6	4	60	150	40	1750	90	10	/85	0		Higher fat (108 calories), insulin unchanged. No change in glycosuria
		7	4	60	120	120	1640	165	30	/90	0.9		Control, gain in tolerance after high fat
		8	4	60	89	150	1640	194	30	/163	20.2		High carbohydrate, better borne after high fat
		9	4	60	138	150	2082	199	30		20.2		Higher fat (442 calories above diet 8)
		10	4	60	180	150	2460	203	30	/172	21.4		Higher fat (820 calories above diet 8); no change in glycosuria
		11	4	60	180	175	2560	228	45		1.3		Increase in carbohydrate with fat high, requires quantitative insulin
		12	5	60	180	200	2660	253	60	/130	2.1		
2	46 M	1	7	66	113	132	1809	181	75	123/186	1.2		Control period
		2	3	66	99	165	1815	213	75	/336	32.3	++	Quantitative excretion, 31 of 32 mgm.
		3	3	66	84	198	1812	244	75	/410	53.3	+++	Quantitative excretion, 21 of 31 grams
		4	3	66	113	132	1809	181	75	/210	3.6		Control diet. Loss of tolerance after high carbohydrate
		5	3	66	143	66	1815	118	75(R)	106/115			Insulin reactions with lower carbohydrate, high fat, same calories
		6	3	66	156	40	1804	94	20(R)	/118			Insulin reactions, with insulin greatly reduced
		7	3	66	113	132	1809	181	55	/120			Control diet, gain in tolerance after high fat
3	47 F	1	4	60	125	150	1965	198	0	160/192	1.3		Control period
		2	3	60	188	150	2530	204	0	155/196	3.0		Fat added; slight increase in glycosuria
		3	3	60	125	150	1965	198	0	176/210	3.8		Control diet, no change in tolerance after high fat
		4	5	60	125	200	2165	248	0	186/290	32.0	++	High carbohydrate, quantitative excretion, 28 of 50 grams
		5	2	60	125	285	2505	333	0	190/313	71.4	+++	Higher carbohydrate, excretion of 39 of 85 grams
		6	4	60	125	150	1965	198	0	/223	4.5		Control diet, no gain, loss of tolerance after high carbohydrate
4	29 M	1	6	76	130	145	2075	202	50	152/166	4.4		Control period
		2	9	76	112	190	2075	245	50	/316	37.7	++	High carbohydrate; quantitative excretion, 33 of 43 grams. No gain in insulin effect
		3	7	76	130	145	2075	202	50	/234	22.1	+	Control diet, loss of tolerance after high carbohydrate, higher blood sugar, more glycosuria
		4	6	76	152	100	2075	159	50	164/183	3.8		Apparent gain in tolerance on high fat
		5	2	76	170	60	2075	121	50(R)	/78			
		6	2	76	170	60	2075	121	30				
		7	2	76	170	60	2075	121	20	/120	1.0		

TABLE V—Continued

Case number	Age and sex	Diet number	Days on diet	Protein	Fat	Carbo- hydrate	Calories	Total glucose value	Insulin	Blood sugar (m.c./p.c.)	Glycosuria	Ketonuria	Remarks
	years			grams	grams	grams		grams	units per 12 hours	mgm. per 100 cc.	grams per 24 hours		
5	38 F	1	7	60	125	80	1685	128	40	218/	1.6		Low carbohydrate
		2	13	60	125	150	1965	198	50	242/218	1.2		Control diet
		3	3	60	188	150	2530	204	50	/198	1.1		No loss of tolerance with high fat added
		4	3	60	125	150	1965	198	50	/205			Perhaps gain in tolerance after high fat
		5	3	60	125	200	2165	248	50	/242	47.3	++	Quantitative excretion, 47 of 50 grams carbohydrate added
		6	3	60	125	235	2305	283	50	/414	68.1	++	Excretion of 21 of 25 grams carbohydrate added
		7	3	60	125	150	1965	198	50	/230	1.2		No gain in tolerance after high carbohydrate
6	44 F	1	14	60	125	150	1965	198	38	/131	14.0		Control period
		2	3	60	188	150	2530	204	38	/136	14.0		No loss of tolerance with added fat
		3	3	60	125	150	1965	198	38	/110	10.7		Control diet, gain in tolerance after high fat, less glycosuria, blood sugar lower
		4	3	60	125	200	2165	248	38	/246	57.1	++	Increase of 50 grams carbohydrate, glycosuria increased 47 grams
		5	3	60	125	285	2505	333	38	/424	102.3	++++	Increase of 85 grams carbohydrate, glycosuria increase of 55 grams
		6	3	60	125	150	1965	198	38	/212	23.4		Loss of tolerance after high carbohydrate. Increased glycosuria and higher blood sugar
9	56 F	1	6	57	98	114	1518	157	25	161/130	5.7		Control period
		2	4	57	67	171	1518	211	25	/152	9.9		High carbohydrate, excretion of 4.2 of 54 grams
		3	4	57	35	245	1518	282	25	/194	15.7		Higher carbohydrate, excretion of 5.8 of 71 grams
		4	4	57	98	114	1518	157	25	/101	1.7		Control diet, gain in tolerance after high carbohydrate
		5	4	57	126	38	1518	84	25	/93	1.0		High fat, low carbohydrate
		6	4	57	98	114	1518	157	25	/146	7.5		Control diet, loss of tolerance following high fat
		7	4	57	98	285	2250	328	25	/245	22.5		Increase of 171 grams in carbohydrate, increased excretion of only 15 grams
		8	4	57	98	285	2250	328	50	/141	3.2		Insulin increase of 25 units. $19.3 \text{ grams} \div 25 = \text{glucose equivalent of } 0.8$
10	68 M	1	7	60	102	120	1638	165	60	134/161	3.1		Control period
		2	3	60	60	217	1648	258	60	/184	13.1		High carbohydrate; increased excretion of only 10 of 93 grams
		3	3	60	40	262	1648	301	60	/192	17.1		Higher carbohydrate; increased excretion of only 4 of 43 grams
		4	3	60	102	120	1638	165	60	152/154	0		Control diet, gain in tolerance, lower blood sugar, less glycosuria
		5	3	60	138	40	1642	89	60	/186	1.0		High fat, blood sugar higher, more glycosuria
		6	3	60	102	120	1638	165	60	/180	3.5		Control diet, loss in tolerance after high fat, higher blood sugar, more glycosuria
11	43 F	1	8	60	125	150	1965	198	50	/138	5.2		Control period
		2	4	60	188	150	2530	204	50	/141	6.4		Added fat, increased glycosuria
		3	3	60	125	150	1965	198	50	/191	6.4		Control diet, higher blood sugar, increased glycosuria after high fat
		4	4	60	125	200	2165	248	50	/158	10.6		High carbohydrate; excretion of only 4.2 of 50 grams
		5	4	60	125	300	2565	348	50	/230	16.8	+	Higher carbohydrate, excretion of 6.2 of 50 grams

CYRIL M. MACBRYDE

TABLE V—Continued

Case number	Age and sex	Diet number	Days on diet	Protein	Fat	Carbo-hydrate	Calories	Total glucose value	Insulin	Blood sugar (a.c./p.c.)	Glycosuria	Ketonuria	Remarks
	years			grams	grams	grams		grams	units per 12 hours	mgm. per 100 cc.	grams per 24 hours		
11	43 F	6	4	60	125	150	1965	198	50	/110	0		Control diet, no glycosuria, lower blood sugar following high carbo-hydrate
12	53 M	1	6	60	125	150	1965	198	40	/68	2.6		Control period
		2	3	60	188	150	2530	204	40	/215	6.7		Increased glycosuria, higher blood sugar with high fat
		3	3	60	125	150	1965	198	40	/132	1.8		Control diet, decreased tolerance after high fat
		4	3	60	125	200	2165	248	40	/232	8.3		High carbohydrate, excretion of 6.5 of 50 added grams
		5	3	60	125	285	2505	333	40	/265	19.2		Excretion of 11 of 85 added grams
		6	3	60	125	150	1965	198	40	/70	0		Control diet, gain in tolerance following high carbohydrate
14	52 F	1	16	100	170	200	2730	265	55	206/110	3.4		Control period
		2	5	100	148	250	2730	323	55	/151	4.9		High carbohydrate; small excretion increase, 1.5 of 58 grams
		3	9	100	128	300	2730	371	55	200/162	6.6		Higher carbohydrate, increased excretion of only 1.7 of 58 grams
		4	8	100	170	200	2730	265	55	/131	0		Control diet, improved tolerance after high carbohydrate, lower blood sugar, less glycosuria
		5	6	100	170	200	2730	265	40	/91	0		Control diet, gain in tolerance permits lower insulin dose
15	55 F	1	18	70	119	140	1911	175/166	4.9				Control period
		2	8	70	88	210	1911	148/163	6.3				High carbohydrate, increased excretion of only 1.4 of 67 grams
		3	7	70	119	140	1911	193	70	/116	0		Control diet, gain in tolerance, lower blood sugar, less glycosuria

MacLeod (20) offers a similar explanation of these phenomena, stating that a lower level of hyperglycemia acts as an adequate stimulus to insulin secretion after sensitization of the secreting apparatus by a previous rise in the blood sugar. It seemed possible, however, as our experiments progressed, that the increased tolerance noted in some of our patients might be due to either of two factors: (1) increased secretion of insulin, or (2) increased sensitivity to endogenous insulin. Abderhalden and Wertheimer (21), and Bainbridge (22) showed that animals on high carbohydrate diets were much more sensitive to insulin than those which were receiving high fat. Hynd and Rotter (23) also noted that hypoglycemic convulsions were more easily produced in animals receiving large amounts of carbohydrate. Those patients who gained tolerance with high carbohydrate intake in this study fell in the relatively insulin-resistant group. It seemed possible that the increased tolerance was due to a better

response to endogenous insulin. Insulin tolerance tests were therefore performed upon several resistant patients after periods upon the control diet containing 2 grams carbohydrate per kilogram body weight, and after high carbohydrate periods on 3 grams per kilogram. Glucose tolerance tests were performed before and after the high carbohydrate as a further check upon the effects of the diet, and because the only other similar curves upon diabetics we have been able to find were the rather inconclusive ones of Watson and Wharton (24). Similar insulin and glucose tolerance studies were made upon several of the sensitive group.

The results of these studies are shown in Table IV. Glucose tolerance curves in two of the three sensitive patients showed a higher percentage rise after the high carbohydrate diets, and in one there was practically no change. In all three resistant patients much lower curves were obtained on the high carbohydrate than on the control diets. In

sulin tolerance curves showed no change in sensitivity in the sensitive patients. A much more marked depression of the blood sugar was evident, however, in the relatively resistant group. This increased sensitivity to insulin was evident whether the insulin was given subcutaneously or intravenously.

While these results do not exclude the possibility of increased insulin production in the relatively resistant group, it seems probable that at least a part of the increased tolerance is due to the patient's greater sensitivity to his own insulin.

The sensitive group, on the contrary, seem rather constantly to respond maximally to endogenous or exogenous insulin, and their response is unchanged by diet. The glucose tolerance, however, is in some of the sensitive patients decreased upon high carbohydrate ingestion. It seems possible, therefore, that in these patients excessive strain upon the pancreatic islets has resulted in a diminished endogenous insulin supply. These patients may be thought of as having diabetes which is primarily pancreatic or insular, since in so many respects their reactions resemble those of Allen's depancreatized dogs.

In the insulin-resistant cases, on the other

hand, evidence indicating pancreatic islet insufficiency is by no means so clear. The gain in tolerance upon high carbohydrate diets, accompanied by a definitely increased sensitivity to insulin, suggests that extra-pancreatic factors decreasing the effectiveness of endogenous insulin may be at least partly responsible for this type of diabetes.

### *Extra-pancreatic factors in diabetes*

Our studies emphasize the fact that we can no longer consider diabetes a unitarian disease, caused solely by an inadequate production of insulin. Warren (25) and other pathologists have demonstrated that the non-diabetic pancreas may reveal changes previously described as the cause of diabetes, whereas the diabetic pancreas may in many instances show no definite disease. On the other hand, studies of recent years have shown that a number of other factors must be considered as exerting profound influences upon carbohydrate metabolism.

There is increasing evidence that the pituitary and adrenal glands may play an important part in the etiology of the common type of clinical diabetes (26). Hyperthyroidism is known to be

TABLE VI  
*Responses to various test diets*

Case number	Age and sex	Diet number	Weeks on diet	Protein	Fat	Carbo- hydrate	Insulin	Blood sugar (u.c./p.c.c.)	Glycosuria	Remarks
	years			grams	grams	grams	units per 24 hours	mgm. per 100 cc.		
1	31 M	1	1 year	70	150	200	75	/120		Fat raised in diet, insulin lowered, fat well tolerated
		2	12	60	180	200	60	/130	++ +	Higher carbohydrate, requires increase in insulin dosage, glycosuria and higher insulin requirement persist even when diet returned to previous level
		3	4	60	180	220	65	/164		
		4	2	60	180	200	65			
3	47 F	1	8	60	125	100	0	/180	++	Carbohydrate decreased because of glycosuria Trial of higher carbohydrate Carbohydrate decreased because of glycosuria Trial of higher carbohydrate No gain in tolerance
		2	4	60	80	70	0	/112	++	
		3	32	60	125	150	0	/196	++	
		4	10	60	125	70	0		++	
		5	2	60	110	120	0	/168	++	
		6	2	60	100	100	0	/170	+	
5	38 F	1	10 years	60	185	50	10	173/	++	No gain in tolerance with increase in carbohydrate; proportional doses of insulin required
		2	10	60	100	80	20			
		3	4	50	50	100	25		++	
		4	2	60	125	150	30	242/218	++	
		5	1	60	125	200	40	/260	++	
		6	3	60	125	150	30	/252	+	



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TABLE VI—Continued

Case number	Age and sex	Diet number	Weeks on diet	Protein	Fat	Carbo- hydrate	Insulin	Blood sugar (a.c./p.c.)	Glycosuria	Remarks
	years			grams	grams	grams	units per 24 hours	mgm. per 100 cc.		
6	44 F	1 2 3 4 5 6 7	4 8 1½ years 8 3 3 2	60 60 70 60 60 60 60	125 125 135 135 140 140 125	150 150 130 130 180 200 150	22 32 32- 39 32 40 32		++	Marked glycosuria and acetonuria with high carbohydrate. Diet 7 same as Diet 2 with practically same insulin requirement. No tendency to gain tolerance
7	53 F	1 2 3	4 4 4	100 100 100	179 134 114	200 250 350	0 0 0	142/205 /160 /212	+++ ++++	
8	33 M	1 2 3	2 years 10 4	70 57 57	150 97 97	125 114 150	30 45 52	138/246 157/173 /194	+	
9	56 F	1 2 3 4 5	4 4 4 4 4	57 60 60 60 60	98 90 100 100 100	114 268 240 300 300	25 35 35 35 30	161/130 /145	+	Gaining tolerance with high carbohydrate, glycosuria and blood sugar practically unchanged on much higher carbohydrate
11	43 F	1 2 3 4 5	8 3 16 1 year 4	60 60 60 60 60	100 125 125 60 50	80 150 150 200 250	10 50 30 30 30	/148 /152	++	Carbohydrate decreased, insulin increased because of glycosuria Higher carbohydrate requires proportional increase in insulin
13	33 F	1 2 3 4 5 6	1 2 8 4 4 20	60 60 60 60 60 60	125 125 150 100 80 60	150 250 150 170 210 300	0 45 30 20 0 0	146/206 /80 /96	++	Enormous increase in carbohydrate permitted, with practically no increase in insulin required
14	53 F	1 2 3 4 5	8 2 2 2 2	100 100 70 70 70	170 170 60 60 60	200 200 225 225 225	55 40 40 35 30	/112 /126		Reduction of insulin with higher carbohydrate Only slight rise in blood sugar and occasional glycosuria with very large increase in carbohydrate
5	55 F	1 2 3 4 5 6 7	6 2 2 2 6 4 2	70 70 70 70 70 70 70	119 89 70 70 70 70 70	140 210 250 250 250 250 250	70 65 65 55 40 30 15	206/110 /115 184/ /105 /140		Gain in tolerance with high carbohydrate until insulin could be omitted entirely
										Gain in tolerance on high carbohydrate; reduction in insulin
										Gain in tolerance as carbohydrate raised; reduction in insulin

accompanied in many cases by decreased sugar tolerance or frank diabetes. Thyroidectomy may diminish the severity of the diabetes and increase the effectiveness of insulin in such patients (27). Claude Bernard's picture directed attention to the importance of the nervous system in carbohydrate metabolism. Injury to or tumors affect-

ing the hypothalamus may cause diabetes. Davi- (28) has been able to produce lesions in the hypothalamus which greatly diminish the severity of the diabetes following pancreatectomy. Animals in which the adrenal medullary tissue has been removed, or the adrenal sympathetic nerve supply has been severed, gain in sugar tolerance

and are hypersensitive to insulin (29). De Takats and Fenn have shown that splanchnic nerve section may increase the effectiveness of insulin in and ameliorate human diabetes (30).

It seems evident that all of the factors mentioned must operate through the liver since it serves as the source of the blood sugar during fasting. Diabetics with various hepatic disorders may require disproportionately large doses of insulin (31). Himsworth (32) concludes from a series of interesting experiments that carbohydrate ingestion increases susceptibility to insulin by causing an increase in an hypothetical insulin activator produced in the liver. The evidence supporting the existence of such a factor is as yet inconclusive.

Present clinical and experimental knowledge indicates that many cases of diabetes may not be due primarily to inadequate production of insulin. The central nervous system, the pituitary, the thyroid, the suprarenals and the liver form a chain of factors influencing the blood sugar level and the storage and combustion of carbohydrate. Extra-pancreatic factors may, by interfering with the action of endogenous insulin, be of importance in the etiology of diabetes.

#### SUMMARY AND CONCLUSIONS

1. The history of the dietary management of diabetes reveals that the greatest students of the disease have differed widely concerning the optimum balance of the various foodstuffs. The fact that many diabetics will gain tolerance on high carbohydrate diets has been reemphasized by recent workers and has attracted much attention. Less attention has been paid to the equally important fact that other diabetics experience deleterious results when allowed large amounts of carbohydrate.

2. A group of diabetics intensively studied over a three year period fall into two classes, the relatively insulin-sensitive and the relatively insulin-resistant. The resistant type tend to be older, frequently are obese, often have hypertension, and are less subject to acidosis and coma. The sensitive type are usually younger, thin, or of normal nutrition, have low blood pressures and a marked tendency to acidosis. The two groups cannot be separated according to severity, since if the insulin

requirement be used as the criterion, the resistant group would seem the more severe, but judged by the tendency to acidosis, the sensitive group would seem to have the more serious type of disease.

3. The insulin-sensitive group failed to gain tolerance on high carbohydrate diets. Only one exception among eight patients was noted to this general rule. Relatively high fat was well borne.

4. The relatively resistant group without exception gained tolerance upon a high carbohydrate intake. In several instances this was shown to be accompanied by increased sensitivity to insulin.

5. Recent studies have shown the probable importance of extrapancreatic influences upon carbohydrate metabolism. It is interesting to note that our insulin-sensitive patients resemble in many respects the partially pancreatectomized animal. They respond well to exogenous insulin, but seem to produce too little of the endogenous hormone. When subjected to the excessive burden of a high carbohydrate intake they may lose tolerance, perhaps as the result of overburdening the damaged or numerically decreased pancreatic islets. Relatively resistant patients, however, react as if the endogenous insulin supply were adequate in amount, but operating under the handicap of inhibiting factors.

6. Studies such as those here described may prove useful in indicating the type of diet which will lead to maximum individual carbohydrate tolerance. The evidence at present indicates that the insulin-resistant type may be expected to gain tolerance with high carbohydrate, while the insulin-sensitive type may either fail to gain or may lose tolerance with excessive carbohydrate ingestion.

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# THE HETEROPHILE ANTIBODIES IN INFECTIOUS MONONUCLEOSIS AND AFTER THE INJECTION OF SERUM<sup>1</sup>

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Recent investigations have shown that the so-called heterophile antibodies found in cases of infectious mononucleosis and after injection of horse serum have distinguishing serological characteristics. These findings may prove helpful or differential diagnosis in certain cases with borderline titers or high titers of sheep cell agglutinins, particularly in children and adults with a history of previous serum injection. The present paper deals with a study of the heterophile antibodies in sera from normal persons, from cases of infectious mononucleosis and from individuals who have received injections of serum. The purpose of this investigation was to define more clearly the differences among these sera and to determine the possibilities for using them in differential diagnosis.

The clinician, in a case of infectious mononucleosis would be especially interested in knowing whether a borderline titer (1:40 or 1:80) of the sheep agglutinins<sup>2</sup> is due to a high normal antibody content or to a low titer due to infectious mononucleosis (1). The clinician may also encounter cases, especially in children, in which a diagnosis of infectious mononucleosis or glandular fever is suspected and in which serum had been administered for active or passive immunization before the disease was contracted. In such cases, the heterophile antibodies can be due either to the injection of serum or to infectious mononucleosis. Cases are sometimes encountered which are injected with antitoxic serum because of a tonsillitis simulating diphtheria or because of a rash similar to that of scarlet fever and in which the later course suggests a diagnosis of infectious mononucleosis. Under such conditions a confirmation of the clinical and hematological findings may be desirable. This is possible only if a serological difference can be found between the antibodies in infectious mononucleosis and those found after the injection of serum.

There are also cases in which some of the features of serum sickness resemble those of infectious mononucleosis, namely, the general adenopathy and the enlargement of spleen and liver.

Various animal blood cells and tissues have been used for the absorption of heterophile antibodies and the results of these absorptions recommended for differential diagnosis. Stuart, Tallman and Brintzenhoff (2) used rabbit erythrocytes. Bailey and Raffel (3) used beef blood corpuscles and also found that horse kidney absorbs part of the antibody from cases of infectious mononucleosis. Davidsohn and Walker (4) used guinea pig and rabbit kidney. Stuart and coworkers (2) found high titers of rabbit agglutinins in serum sickness as compared with infectious mononucleosis. Bailey and Raffel (3) found high hemolysin titers for the beef cells in the latter disease.

In the present study, the blood corpuscles of the horse, goat, guinea pig, pig, dog and chicken were used for absorption in addition to the ones employed by the previous investigators, and the titers for all these agglutinins were determined. The horse blood corpuscles were used for absorption because the titer of agglutinins with these cells was found to be high in infectious mononucleosis, a finding noted by Stuart, Griffin, Wheeler and Battey (5) since this work was completed. The pig cells were used for absorption because they were found by Deicher (6) to absorb the sheep agglutinins after injections of serum. Goat erythrocytes were employed because of their antigenic relationship to sheep cells.

## METHODS

*Titration of agglutinins* was carried out approximately after the method used by Stuart et al. (7) for the reasons which he outlined. Five tenths cc. of serum dilutions, beginning with 1:2.5 or 1:5, were made with saline and 0.5 cc. of 1 per cent suspension of blood corpuscles was added, the tubes shaken, incubated at 37° C. for about

<sup>1</sup> This study was carried out under a special grant from the Friedsam Foundation.

<sup>2</sup> The terms sheep agglutinins, beef agglutinins, etc., are frequently used in this article as a substitute for more cumbersome, though perhaps more exact terms, such as, sheep red blood cell agglutinins, beef red blood cell agglutinins, etc.

2 hours and read after storage in the ice box over night. The titer was read as the "final dilution" of serum in which agglutination was observed with the naked eye.

*Hemolysins for beef cells* were determined only in a few cases, after the method of Bailey and Raffel (3), but with half the amounts of all components: 0.5 cc. of serum dilution, 0.25 cc. of 2 per cent suspension of beef blood corpuscles and 0.25 cc. of guinea pig serum 1:10 for complement. Due to the presence of hemolysins in the dilution of the guinea pig serum used, a control tube containing complement and beef cells alone was used. A 1:30 or 1:60 dilution of the complement, which did not hemolyze by itself, occasionally did not seem to provide sufficient complement.

Absence of agglutinins or hemolysins in the first dilution used is recorded as "0" in the tables.

*Absorption tests* were also carried out after the method of Stuart et al. (2). The third absorption was found to make no appreciable difference in titers as compared with the second. Therefore, only 2 absorptions were carried out in the later part of this study. The sera of approximately the same number of cases of infectious mononucleosis, and after the injection of serum, were studied with 2 and with 3 absorptions, made with blood cells from 5 different species, namely, those of sheep, rabbit, beef, horse and pig. The sera were titrated for agglutinins with each of the different kinds of blood corpuscles before and after each absorption. Control sera were absorbed only twice, and the serum was used in a dilution of 1:2 because of the low titers.

#### SUBJECTS

Sera were obtained from 9 cases of infectious mononucleosis in different stages of the illness.<sup>3</sup>

The sera of 24 patients were studied after the injection of therapeutic serum. The sera injected were: in 13 cases, antitoxic scarlet fever serum; in 4 cases, diphtheria antitoxin; in 5 cases, antimeningococcus serum and in 2 cases, antigas gangrene and antitetanus serum combined. The patients' sera were chosen without regard to the presence or absence of serum sickness.

Sera from normal healthy individuals and from persons who were suffering from conditions other than infectious mononucleosis were studied for comparison. None of these subjects gave a history of previous serum injection.

TABLE I

*Control sera from cases without infectious mononucleosis and not previously injected with serum*

Number of control sample	Agglutination titer with blood corpuscles of *								Hemolysin titers for beef blood corpuscles
	Sheep	Beef	Goat	Horse	Pig	Rabbit	Dog	Guinea pig	
1	0	0	10	80	40	40	20	20	0
2	10			0	40	40	40	20	40
3	0	0	10	5	40	20	40	20	0
4	0	0	10	0	20	20	10	0	0
5	0	0		0	40	20	20	0	20
6	10	0		0	20	10	0	0	0
7	0	0	0	10	20	20	?	0	0
8	0	0	0	10	20	20	?		0
9	5			20	20	80	20	20	
10	20			20	80	160	40	40	
11	20	5		10	80	40	40	40	80
12	10	0		10	40	20	40	20	40
13	10	0		10	40	20	20	20	20
14	0	0		40	40	40			
15	10	0		20	40	40			
16	10	0		10	40	80			
17	0	0	10	10	20	20			
18	20	0	40	20	80	40			20
19	20			10	40	40			
20	0	0		10	20	20			
21	20	10?		20	80	40			
22	0	0		40	20	20			
23	10	0		20	80	40			
24	0	0		0	20				
25	0	0		0	0	10			
26	0	5		10	20	20			
27	10	5		10	20	20			0
28	0	0		0	20	20			0
29	0	0		0	40	20			
30	0	0		20	20	40			
Average titer	6.8	1.0	11.4	13.8	36	35.2	22.3	16.7	15.7

\* Figures 5, 10, 20, etc., to indicate a titer of 1:5, 1:10, 1:20, etc.

the average titer of agglutinin for the various kinds of blood corpuscles, and the same sequence was usually observed in the individual sera. The agglutinins for beef blood corpuscles showed the lowest titers and were usually absent in the dilution 1:5 or 1:10. The sheep agglutinins were present in somewhat higher titer. The highest titers were obtained with rabbit and pig corpuscles. The approximate sequence of the agglutinability was as follows: beef, sheep, goat, horse, guinea pig, dog, rabbit, pig.

#### *Titration in cases injected with serum (Table II)*

There was a marked increase in the beef hemolysins in most of the sera. The sequence of the average agglutinin titers for the different kinds of

TABLE II  
Sera from cases injected with serum

Case number	Days after injection	Agglutination titer with blood corpuscles of								Hemolysin titer for beef blood corpuscles
		Sheep	Beef	Goat	Horse	Pig	Rabbit	Dog	Guinea pig	
1	11	320	10		40	640	320			
2	11	40 to 80	0		80	320	80			
3	17	160 to 320	5		40	320	80			
4	12	160	10		160	640	160			
5	12	80	5		40	160	80			
6	10	40 to 80	10		160	320	320			
7	13	80	5				320			
8	9	320	10	320	640	1280	640	160	640	2560
9	11 and 15	160	20		5120	640	640			
10	15	80	20		1280	60	320	320		
11	19	40	0		320	80	160	80		
12	11	40 to 80	5	400	640	320	640	320		
13	9	160	0	160	640	320	320			1280
14	13	80	0		80	160	160			
15	9	160	40	320	1280	320	640	320	160	160
16	9	160	40		640	80	160			
17	29	160	40		160	640	160	640	160	640
18	14	160	80		320	1280	1280	640	320	640
19	11	80	40		80	1280	1280	640	320	1280
20	8	160	80		160	2560	1280	1280	1280	
21	20	20	10		10	40	40			
22	12	5	0	20	20	20	40	20	20	0
23	20	40	10		80	80	40			
24	14	20	10		40	20	40			
Average titer		113.5	18.8	244	523	507.8	353.3	460	414.3	980

blood corpuscles is as follows: beef, sheep, goat, rabbit, guinea pig, dog, pig, horse.

With two exceptions, namely for horse and rabbit, the sequence of the agglutinins for the different kinds of blood corpuscles is similar to that recorded in normal sera. The increase in agglutinins for 5 of the 8 kinds of cells used, namely those for sheep, beef, goat, guinea pig and dog ranged about 20 times that of the normal value. The increase for pig and rabbit agglutinins is not as great. The horse agglutinins showed the greatest increase—about 40 times normal. This greater increase in horse agglutinins occurred only in certain cases, while in others the increase was similar to that noted with other cells.

The smaller increase in the pig and rabbit agglutinins may be due to the normally higher titers of these antibodies as compared with agglutinins for other cells.

These results together with the observations on the absorption tests, to be mentioned later, suggest that the agglutinins found after the injection of serum may be due to an increase in normal agglutinins for most cells (8) and that the increase

in the horse agglutinins may be a more specific reaction.

#### *Titration in infectious mononucleosis (Table III)*

A marked increase in sheep agglutinins in the serum of cases of infectious mononucleosis was first noted by Paul and Bunnell (9) and later by other investigators (8, 10), and this is now used routinely as an aid in diagnosis. Increases in beef hemolysins (3) and more recently in horse agglutinins (5) have also been noted in this condition. These findings are here confirmed. In addition, an increase in goat agglutinins was observed in 5 of our cases.

TABLE III  
Sera from cases with infectious mononucleosis

Case number	Agglutination titer with blood corpuscles of								Hemolysin titer for beef blood corpuscles
	Sheep	Beef	Goat	Horse	Pig	Rabbit	Dog	Guinea pig	
1	1280	0		2560	160	160	80		
2	320	0		640	40 to 80	80	40		
3	160	0	160	160	40 to 80	80			1280
4	640	0	1280	2560	40	40	20	20	1180
5	320	0	320	800	40	40	20	10	640
6	320	10	320	640	40	40			
7	2560	0	2560	2560	80	40	40	40	1280
8	640	0		640	40	40	Under 50	Under 50	
9	160/320	5		640	20				
Average titer	711	1.07	928	1254	55.5	60	41.7	30	1535

The titers for all of these agglutinins are about 80 to 100 times the normal value and about 2 to 6 times the value found after injection of serum. Occasional cases show high titers after serum injection which are comparable with the low titers seen in some cases of infectious mononucleosis. The difference in the titer alone, therefore, cannot be used for differential diagnosis.

The agglutinin titers for sheep, goat and horse blood corpuscles together with the beef hemolysin titers are very high in contrast to the low titers of agglutinins for rabbit, pig, dog, and guinea pig. The average titer of the latter agglutinins is not more than twice the normal. The difference between the agglutination in infectious mononucleosis and after the injection of serum is striking. After the injection of serum, there is an increased titer for all these kinds of blood corpuscles, whereas, in infectious mononucleosis, the increase is limited to antibodies against certain blood cor-





several examples of partial receptors (11) common to 2 species of blood corpuscles.

*Absorption in sera from cases injected with serum*  
(Table IVb)

Absorption was carried out for sheep agglutinins with beef and rabbit cells in 21 cases. Absorption of the 3 other agglutinins investigated in the control cases was performed with sheep, rabbit, horse, beef and pig blood corpuscles in a number of cases. Only a few experiments were carried out with goat, guinea pig and dog blood corpuscles. The results may be summarized in the same order as noted in the controls.

Sheep agglutinins are absorbed in about the same manner, but to a greater degree than in controls.

Rabbit agglutinins are absorbed by different corpuscles in the same manner as from control sera.

As in the controls, horse agglutinins are little absorbed by sheep and beef blood corpuscles, but there is a distinct difference from the controls in the absorption with pig and with rabbit cells. The former absorb less and the latter more of the horse agglutinins. In half of the cases more than 90 per cent of the horse agglutinins were absorbed by rabbit cells.

The absorption of the pig agglutinins by rabbit and beef blood corpuscles is the same as in controls.

The results obtained with the absorption of the beef agglutinins by different blood corpuscles are not so valuable because of the low titer of these agglutinins. The goat and sheep blood corpuscles absorb the agglutinins for each other (both blood corpuscles have the same receptors in contrast to rabbit and pig blood corpuscles). The goat blood corpuscles do not remove the horse agglutinins. Guinea pig and dog blood in a few experiments did not show any striking features. The same was true for chicken blood which is not recorded here.

*Absorption in sera from cases of infectious mononucleosis* (Table IVc)

Absorption was done with sheep blood corpuscles in 5 cases, and with the other 4 kinds of blood corpuscles used in the controls in 9 cases.

The result is less complicated than in normals and in cases injected with serum.

In general, the agglutinins for each of the cells that are increased in titer are absorbed by any of the cells in the same group. For example, beef blood corpuscles absorb the sheep agglutinins completely, but they also absorb the horse and goat blood agglutinins almost completely. The beef hemolysins were not examined. Blood corpuscles of all these species seem to possess one common receptor for the increased agglutinins and hemolysins. In this respect, the antibodies in infectious mononucleosis differ from those in controls and in cases after injection of serum. The absorptions of sheep and goat agglutinins by their blood corpuscles are similar in infectious mononucleosis and after injection of serum, and are probably the same as in controls.

The blood corpuscles of rabbit, pig, guinea pig, dog and chicken (not recorded in the table), for which the antibodies are not increased, react quite differently. They do not absorb any appreciable amount of the increased agglutinins for the sheep, horse and goat cells. The amount of agglutinin absorbed in the "increased group" is often less than in normal sera and in sera from cases injected with serum. The sheep agglutinins, which in normal sera and in sera after injection of serum are absorbed to a great extent by the different kinds of blood corpuscles, are very little absorbed by rabbit and pig blood corpuscles in infectious mononucleosis. This difference was previously noted by Stuart et al. (2) for rabbit blood. This fact may be explained by the higher resistance of the increased antibodies to absorption by nonspecific antigens.

The absorption of the agglutinins which are not increased, such as rabbit and pig agglutinins, is similar to that found in the controls and after injection of serum.

*Application of results for differential diagnosis*

In applying the absorption tests for differential diagnosis, the specific and mutual absorption of the increased antibodies by their antigens makes it possible to differentiate sera of cases of infectious mononucleosis from those of both normal cases and those injected with serum. Each of these antigens may be used. In making a

choice between the antibodies to be used for this purpose, it is necessary to select an antibody which is not absorbed to a great extent in normals and in cases injected with serum. The sheep agglutinins, for example, are not useful because they are largely absorbed by all blood corpuscles, and especially by beef blood corpuscles, in normals and cases injected with serum (cf. Table IVa and b and Stuart (12)). Among 21 cases injected with serum, the sheep agglutinins were completely removed in 3 and over 90 per cent removed in 3 others. Absorption with boiled beef blood corpuscles in 3 of our cases showed about the same amount of absorption as with raw blood corpuscles. Hence, Bailey and Raffel's method (3) of determining the sheep agglutinins after absorption with beef cells is not useful for the differential diagnosis.

If the sheep cells are to be used for differential diagnosis, the antigen selected for absorption must be of the group in which antibodies are not increased in infectious mononucleosis, such as rabbit, guinea pig, pig and dog corpuscles. When this is done, the titer of sheep agglutinins from cases of infectious mononucleosis will be found almost unaltered, whereas in normals or after the injection of serum it will be reduced after absorption. Stuart et al. (2) used rabbit blood corpuscles as absorbing antigen. Davidsohn and Walker (4) used rabbit kidney and guinea pig kidney, but he had a high degree of nonspecific absorption which obscures the results. The results of the absorption with rabbit blood corpuscles in infectious mononucleosis agree with those of Stuart et al. (2), but there are a few cases injected with serum in which the sheep agglutinins are not absorbed as well by rabbit blood corpuscles. In Table Va are shown examples of cases in which absorption tests gave doubtful results.

Since, as shown in Table IVc, the horse agglutinins are almost completely absorbed by beef blood corpuscles in infectious mononucleosis and are particularly high in infectious mononucleosis and after injection of serum, beef blood corpuscles were selected for absorption and the horse agglutinin titer was determined. Though applied in only 10 cases, this method appears to be somewhat more practical than the others. Cases il-

TABLE V

*Results of absorption experiments in certain cases illustrating their use in differential diagnosis*

(a)				(b)			
Case number*	Sheep agglutinins present after absorption with blood corpuscles of rabbit			Case number	Horse agglutinins present after absorption with blood corpuscles of beef		
	After 1st absorption	After 2nd absorption	After 3rd absorption		After 1st absorption	After 2nd absorption	After 3rd absorption
	per cent	per cent	per cent		per cent	per cent	per cent
SERA FROM INFECTIOUS MONONUCLEOSIS							
1	75 or more	75 or more	75 or more		In no case was more than 3 per cent of the agglutinin present after the first absorption		
2	100	100	50				
3	100	75					
Cases 4 to 9 no absorption							
SERA FROM SUBJECTS INJECTED WITH SERUM							
5	50	50		13	100	50	50
7	66	33		15	100	50	50
8	66	66		18	25	25	
15	75	50	50	20	50	50	
In the remaining sera there was less agglutinin present after absorption than in these sera				In the remaining sera there was more agglutinin present than in any of these sera			
CONTROL SERA							
21		100			More than 50 per cent of agglutinin present in every instance		
18		50					
12		50					
In the remaining sera there was less agglutinin present after absorption than in these sera							

\* The case numbers in the three groups refer to case numbers in Tables III, II, and I respectively.

illustrating the use of this test in differential diagnosis are recorded in Table Vb. The beef-horse method has the additional advantage that only one absorption seems to be necessary.

#### *Interference of heterophile antibodies in patients with infectious mononucleosis injected with serum*

Stuart et al. (2) report only one case of infectious mononucleosis where they found an increase of the rabbit agglutinins from 1:160 to 1:1280. This case had been injected previously with 0.1 cc. of horse serum. He points out, that "it is questionable, whether this amount of serum could have initiated the rise."

We had an opportunity to observe a case of the same kind.

*Case 1.* An 18 year old girl showed a typical picture of infectious mononucleosis; slight fever, ulcerative tonsillitis, general enlargement of the lymph nodes and the spleen just palpable. The white blood count after admission to the hospital was 10,200, with 18 per cent polymorphonuclear leukocytes, 14 per cent large and 61 per cent small lymphocytes, 1 per cent eosinophiles, 1 per cent basophiles and 5 per cent monocytes.

The titration of the serum with the different kinds of blood corpuscles showed:

	Blood corpuscles				
Agglutinins for...	Sheep	Beef	Horse	Pig	Rabbit
Titer.....	640	<10	2560	20	40

The titration value did not change during the normal course of the illness, blood samples being taken on the 4th and 9th day after admission. The patient was discharged the 13th day after admission. On this day 0.1 cc. of diphtheria antitoxin was injected intradermally to investigate a possible sensitivity to horse serum caused by the infectious mononucleosis antibody. There was only a slight cutaneous reaction to the serum. Seven days after the injection, the patient had an unusually severe local serum exanthem at the site of the injection followed by a severe serum sickness with high fever, so that she was again admitted to the hospital. The titration of the serum at this time showed:

	Blood corpuscles				
Agglutinins for...	Sheep	Beef	Horse	Pig	Rabbit
Titer.....	640	<10	2560	20	160

8 days later or 15 days after the injection:

	320	<10	2560	40	640
--	-----	-----	------	----	-----

The only difference in the titers was the marked increase of the rabbit agglutinins. All other agglutinins, including those for pig cells which are usually high after the injection of serum, remained practically unchanged. The absorption tests showed but a slight change in the sheep agglutinins after the absorption with rabbit blood corpuscles; in this respect the sheep agglutinins retained the characteristic observed in infectious mononucleosis. Similarly, beef blood completely absorbed the sheep agglutinins, as was found before the injection of horse serum. Distinct changes occurred in the absorbability of horse agglutinins after the injection of serum. As seen in Table VI they became less absorbable by sheep blood corpuscles and non-absorbable by beef blood corpuscles.

TABLE VI

*Absorption tests in a case of infectious mononucleosis injected with horse serum*

	Horse agglutinins present after absorption with blood corpuscles of	
	Sheep	Beef
a. 4 days before injection of serum.....	per cent 6.2*	per cent 1.6†
b. 7 days after the injection....		12.5†
c. 15 days after the injection....	50†	100†

\* After second absorption.

† After third absorption.

*Case 2.* This patient received an injection of horse serum (antitoxic scarlet fever serum) because of a severe scarlatiniform rash. The rash turned out to be a symptom of infectious mononucleosis. The serum of this patient examined 11 days after the injection did not show any change in the titer or absorbability of the different agglutinins. Possibly, in the first period of the illness the antibody forming system does not allow the formation of a second antibody.

*Case 3.* A 24 year old nurse suffered from a sore throat which looked like a streptococcic sore throat, but diphtheria bacilli were cultured from the throat. She was, therefore, injected with 40,000 units of diphtheria-antitoxin. Twelve days later she developed a serum sickness, consisting of urticaria and enlarged lymph nodes, especially in the neck. About 20 days after the injection the adenopathy persisted and, in addition, 37 per cent mononuclear cells were found in her blood smear. Twenty-eight days after the injection of serum the white blood count was 9,500, with 56 per cent lymphocytes, partially large, and 4 per cent monocytes. Later, the patient contracted a subacute nephritis. Titrations of her serum are shown in Table VII.

TABLE VII

*Titrations of serum in Case 3*

Days after serum injection	Titer of agglutinins for blood corpuscles of				
	Sheep	Beef	Horse	Pig	Rabbit
26	40		160	160	80
44	20		320	80	40
52	80	5	80	40	40

Since all the titers were low and the horse or sheep agglutinins not distinctly higher, the result was not particularly useful in differential diagnosis. The absorption tests were also inconclusive. Absorption of the sheep agglutinins by rabbit blood corpuscles was doubtful and only 50 per cent of the horse agglutinins were removed

by the beef blood corpuscles. The stability of the horse agglutinins is not evidence against the diagnosis of infectious mononucleosis when serum has been previously administered (cf. Case 1).

It appears, therefore, that when the two reactions interfere with one another the results of these tests may not be conclusive for differential diagnosis. Further observations may point to some other difference which will be useful in such cases.

#### DISCUSSION

The results of this investigation indicate that the antigen in infectious mononucleosis which reacts with the increased antibodies, is present in goat blood corpuscles as well as in horse, beef and sheep blood corpuscles. The interrelationship of goat, sheep and beef blood corpuscles was shown by Ehrlich and Morgenroth (11). The antibodies in infectious mononucleosis are probably not the same as those of Weil (13), as suggested by Stuart et al. (5), since they react with horse blood corpuscles, as shown in this study, are absorbed by horse tissue and since the antibodies described by Weil are not absorbable by horse kidney. Our absorption experiments with the different kinds of blood corpuscles show that each species of blood corpuscles containing this antigen removed almost completely the agglutinins for each of the other blood corpuscles in this group. The conclusion seems to be justified that we are dealing with one specific antibody and also with one antigen which is contained in all these blood corpuscles. While this antibody has certain characteristics in common with the Forssman antibody, it has other properties which are quite different (3, 14). The source of this antibody remains unknown. It is probably related to the etiologic agent in infectious mononucleosis.

The antibodies after the injection of serum are more difficult to explain. Our findings suggest that they represent an increase in the normal agglutinins. Therefore, the Forssman character of the sheep antibodies after the injection of serum is not surprising, for the normal sheep antibodies are of Forssman character (14, 15). The antibodies consist of agglutinins and hemolysins for a large variety of blood corpuscles, many of which

can be separated by absorption. They do not react with any common antigen, as is the case in infectious mononucleosis. There is nothing to suggest that some are "major" and others "minor" agglutinins, as in the case of certain bacterial species. The antigen which is agglutinated to the highest titer, namely, horse blood corpuscles, absorbs less of the rest of the agglutinins than any other antigen. Unless an antigen is found which absorbs or neutralizes all the increased antibodies, the increase after the injection of serum must be considered as nonspecific.

This assumption does not exclude the fact that some of the antigens with which the antibodies after the injection of serum react, contain a receptor for one or more different antibodies. Thus, rabbit and beef blood corpuscles absorb and, therefore, contain a receptor for pig agglutinins; rabbit blood corpuscles may contain a receptor for horse agglutinins and the pig blood corpuscles contain one for sheep agglutinins.

A more specific nature of the antibodies after the injection of serum was recently suggested by Stuart et al. (5). Because of the absorption of sheep lysins by raw and boiled beef and by rabbit blood corpuscles and because of the inhibition of the beef lysins by horse serum, Stuart concluded that there is a thermostable antigen common to these three which may cause the increase of beef and of some other agglutinins. This is, of course, different from the antigen in infectious mononucleosis for it is contained in rabbit blood corpuscles. We suppose that Stuart assumed that the reaction after the injection of serum is due to the action of several antigens of this kind (as for instance the Forssman antigen) which are supposedly contained in horse serum. The above mentioned antigen cannot be responsible alone for the numerous antibodies which are not absorbed by this one antigen. In contrast to this theory, we consider the reaction after the injection of serum as nonspecific, with certain restrictions mentioned above, because there is no mutual absorption of the antibodies and because of the resemblance to the normal antibodies.

#### SUMMARY

1. In infectious mononucleosis, the antigen with which the so-called heterophile antibodies react, is

found in horse and goat blood corpuscles as well as in sheep and beef blood corpuscles. The agglutinins for 3 of these species of blood corpuscles, namely, those of sheep, horse and goat are markedly increased. In the case of beef blood corpuscles the hemolysins are increased, but the agglutinins are not. Each of these kinds of blood corpuscles can absorb almost completely the agglutinins for the blood cells of each of these species. On the other hand, these antibodies are almost non-absorbable by any antigen for which the corresponding agglutinins are not increased.

2. After injection of serum, the increased agglutinins for many kinds of mammalian blood corpuscles show about the same sequence in their titer as is found in sera of persons without a history of injections of serum.

3. Serum from cases with infectious mononucleosis can be differentiated from normal sera and from those obtained after the injection of serum in one of 2 ways: (a) by absorption of the sheep, horse and goat agglutinins with one of the following species of blood corpuscles: sheep, beef, horse and goat; (b) by the resistance of these increased agglutinins to absorption by any of the blood corpuscles the agglutinins for which are not increased in infectious mononucleosis, namely, rabbit, pig, dog and guinea pig.

The most reliable method besides the absorption of sheep agglutinins by rabbit blood corpuscles (Stuart) is the absorption of horse agglutinins by beef blood corpuscles. By the first method, one obtains almost no absorption of the agglutinins in infectious mononucleosis; by the second, almost complete absorption. In normal sera and in sera after the injection of serum the reverse is true.

4. The sera in two cases of infectious mononucleosis were studied after injection with horse serum. In one of these cases the interference of the antibodies characteristic of infectious mononucleosis and those usually found after the injection of serum is shown. A third case is described which illustrates that after the injection of serum the differential diagnosis of infectious mononucleosis cannot always be made serologically.

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# THE INFLUENCE OF CHANGES OF ABDOMINAL TENSION UPON PULMONARY FUNCTION

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## EXPERIMENTAL

Salkin (1) has recently studied normal physiological changes which occur in the abdominal cavity. He has described a process of adaptability in the abdominal muscles which tends to prevent changes in intra-abdominal pressure, and which he terms "abdominal accommodation." He considers this process to be a reflex mechanism dependent upon an intact neuromuscular system, and points out the clinical bearing on intraperitoneal conditions, and functional interrelationship between the pleural and peritoneal cavities. Previous attempts to obtain measurements of abdominal pressure have been accompanied by difficulties (2, 3, 4). When fluid is injected at a certain rate into the abdominal cavity hyperbolic curves are obtained in which the pressure rise is slow at first and then becomes more rapid.

Although the physiology of the intra-abdominal pressure and tension of the abdominal wall has been studied in considerable detail, its relation to pressure within the pleural cavity has been insufficiently considered.

Recent clinical observations by Gordon (5) have indicated the importance of change in abdominal pressure upon function of the thorax. He found that elevation of intra-abdominal pressure during pregnancy apparently influenced the course of chronic pulmonary diseases. In cases of chronic tuberculosis and bronchiectasis, cough, shortness of breath, and excessive sputum were diminished. He attributed the improvement to an increase of the intra-abdominal pressure, which in turn caused elevation of the leaves of the diaphragm.

It has also been shown by Alexander and Kountz (6) that the vital capacity of an individual with chronic pulmonary emphysema may be improved by the application of an abdominal belt, which is believed to increase the intra-abdominal pressure and the tension of the abdominal wall.

To study the influence of abdominal conditions upon intrabronchial and intrapleural pressure, experiments were made upon animals. A bronchoscope was introduced, small hollow copper tubes were inserted into the lower bronchus on each side and were arranged by means of interchangeable tips to occlude the bronchus except for its connection with the lumen of the tube. Rubber tubing was connected to the copper tubes and, by means of a tambour and lever, records on a moving drum were made. Because of secretion, which was partially controlled by atropine, and because of slight movements of the tips, precautions were necessary to assure free excursion with each respiration.

An important part of the study was to determine the effect of cough. This was induced by blowing cigarette smoke directly into the trachea of the partially anesthetized animals. Anesthesia, which consisted of morphine in doses of one and one-half grains with ether only sufficient to permit the passage of the bronchoscope, did not abolish the cough reflex. Measurements of intratracheal, intrapleural, and intra-abdominal pressures could thus be recorded following cough under varying conditions.

For determination of intrapleural pressure two large number fourteen needles were inserted into both pleural cavities, connected to water manometers, and records made upon the moving drum. To record the pressure within the abdominal cavity, a rubber tube with a small balloon attached to its end was introduced through the esophagus into the stomach. This was connected to a mercury manometer, and the balloon distended by air pressure to ten millimeters of mercury.

The effect of both costal and abdominal types of breathing was recorded. Since respiration in the dog is chiefly costal, it was necessary for the production of abdominal breathing to place a binder



about the chest. Later, for the study of the costal type of respiration, the binder was removed and pressure was exerted on the abdominal wall.

To study the effect of abdominal conditions upon the ejection of fluid from the lower bronchi, x-ray studies were made to learn the time necessary to discharge from the lungs, oil opaque to the x-rays. Under the fluoroscope, tubes were introduced and five cubic centimeters of opaque oil were injected into the bronchus of each lower lobe. X-ray pictures were taken at the time, and subsequently at intervals of three days until all the oil had disappeared.

A factor other than abdominal pressure which may significantly influence abdominal thoracic relationships is the position of the diaphragm, which acts to change the relative size of the two cavities. To study this, two types of operation were performed. In one group of animals the chest was opened, and the diaphragm was attached high on one side by suturing it to the chest. The abdominal cavity was thus increased in size at the expense of the thorax without greatly altering the function of either the thoracic or the abdominal walls. In the other type of operation, the abdominal cavity was opened and the diaphragm attached low by suturing it to the abdominal wall, thus artificially increasing the size of the thoracic cavity and diminishing the abdominal. These operations were done on the left side only. The animal was allowed to recover. From four to six weeks later observations were again made of the bronchial pressure, intrapleural pressure, and of the time that it took to empty oil from the lung.

The normal dog has a much more mobile mediastinum than man, and when the position of the diaphragm was changed on one side a shift of the septum and heart was noted. In the experiments in which the diaphragm was attached low, the mediastinum shifted to that side; when it was attached high it shifted to the opposite side. This change does not usually occur in man because the mediastinum is more rigid. It was therefore necessary in these experiments to prevent the shifting of the mediastinum in order to approximate more closely the state of the human chest. This was done by injecting ten per cent emulsion of ground glass in mineral oil into the mediastinum and waiting for a period of four weeks before studies were made. In these animals there was little or no

shifting of the mediastinum when the position of the diaphragm was changed by operation. Experiments were performed on a group of twenty animals prepared in this way.

Since it has been demonstrated that abdominal tension and adaptability of the abdominal wall are dependent upon an intact neuromuscular mechanism (1), the abdominal muscles were resected in some animals. In others, nerve section of the anterior roots from the fourth thoracic to the sixth lumbar was performed. In such animals a marked distention of the abdomen occurred. Adaptability of the abdominal muscles to changes in intra-abdominal pressure was lost. With these procedures it was possible to determine the effect of changes in abdominal pressure and in the position of the diaphragms upon intrabronchial and intrapleural pressures, and upon the drainage of fluid from the lower bronchi.

## RESULTS

*Intra-abdominal pressure.* Measurements of abdominal pressure in animals were found to be rather unsatisfactory. With normal respiration, pressures varied from 0 to  $-3$  to  $-4$  cm.  $H_2O$ . With induced cough, pressures rose from 20 to 30 mm. Hg. In animals with a low diaphragm and a normal abdominal wall the pressure was from 0 to  $-2$  cm.  $H_2O$ , while during cough the pressure reached higher levels than when the diaphragm was in the normal position. In those with high attachment of the diaphragm the abdominal pressure was usually slightly lower than in the normal animal, especially during cough.

While breathing quietly animals whose abdominal wall had been weakened either by cutting the nerves or by dissection of the muscles showed little variation in abdominal pressure from those with intact muscles. The increase on coughing, however, was much less, the manometer readings ranging between  $+10$  and  $+15$  mm. Hg.

Measurements of the intrapleural pressure invariably showed approximately normal values in normal animals and in the ones with normal mediastinum, regardless of the position of the diaphragm. This was not the case, however, when the mediastinum had been fixed previous to the operation, for in such animals, compensation for the change in size of the chest cavity on the operated side could not occur.

TABLE I  
Normal dogs

Dog	Left side				Right side			
	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time for oil	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time of oil
	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days
1	-4 -6	+40	+3 -3	15	-4 -6	+40	+3 -3	18
2	-5 -8	+50	+2 -4	17	-3 -5	+50	+2 -4	15
3	-8 -10	+45	+1 -6	18	-4 -6	+46	+1.5 -6	18
4	-6 -8	+62	+2 -4	19	-4 -6	+58	+2 -4	19
5	-4 -6	+40	+3 -5	19	-4 -6	+40	+3 -5	19
6	-4 -5	+35	+2 -4	19	-4 -5	+39	+2 -5	20
7	-5 -9	+40	+2 -5	19	-5 -9	+37	+2 -5	20
8	-4 -7	+46	+1 -3	18	-4 -7	+43	+1.5 -3	20
9	-4 -7	+54	+1 -4	18	-5 -7	+55	+2 -3	19
10	-3 -8	+48	+1 -4	21	-4 -8	+42	+2 -4	19
11	-6 -10	+51	+2 -5	15	-6 -10	+50	+3 -5	21
12	-5 -8	+44	+2 -4	21	-5 -10	+50	+2.5 -5	19

DIAPHRAGM ELEVATED ON LEFT SIDE, MEDIASTINUM FIXED

Dog	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time of oil	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time of oil
	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days
1	-5 -12	+30	+2 -3	31	-2 -4	+50	+4 -3	17
2	-4 -10	+35	+2 -8	23	-3 -5	+53	+4 -4	18
3	-8 -10	+40	+3 -8	27	-2 -4	+58	+3 -6	19
4	-6 -12	+36	+2 -6	32	-3 -4	+52	+4 -4	15
5	-7 -10	+50	+2 -6	28	-4 -5	+48	+6 -5	18
6	-4 -8	+32	+2 -7	27	-2 -5	+48	+4 -4	19

DIAPHRAGM LOW ON LEFT SIDE, MEDIASTINUM FIXED

Dog	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time of oil	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time of oil
	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days
7	-2 -6	+60	+4 -5	15	-4 -6	+57	+2 -3	23
8	-2 -6	+56	+3 -2	17	-6 -8	+60	+3 -5	23
9	-1 -3	+62	+5 -2	16	-5 -6	+55	+2 -4	21
10	-1 -4	+55	+4 -3	17	-7 -10	+54	+1 -2	22
11	-6 -8	+55	+3 -3	18	-6 -9	+50	+2 -2	22
12	-2 -3	+52	+3 -2	15	-5 -9	+56	+3 -2	21

**Intrapleural pressure.** With low attachment of the diaphragm, intrapleural pressure on the affected side was elevated during respiration to approximately that of the atmosphere, ranging between +1 to -2 cm. of water. This contrasted with a pressure of -4 to -6 cm. of water on the unaffected side or in an intact animal.

In animals with the diaphragm attached high and the mediastinum fixed, the intrapleural pressure was found to be more negative, -6 to -10 cm. of water. Weakening of the abdominal wall tended to make the intrapleural pressure more nearly atmospheric than had been observed previously in the same animal.

**Emptying time of fluid from bronchi.** In normal animals our experience with opaque oil confirmed that of Carlson et al. (7) and his coworkers, who found that oil was expelled from the lungs in from 18 to 21 days. In animals with a diaphragm attached low and with a normal abdominal wall, the oil was expelled from the bronchus of the operated side in 15 to 17 days, whereas

on the opposite side it remained for 22 days. In animals with a diaphragm attached high the oil was expelled in about four weeks, whereas on the opposite unoperated side the normal time of 19 days was usually observed. In animals with a weakened abdominal wall, the time of expulsion was greatly prolonged, lasting approximately 30 days.

TABLE II  
Normal abdominal wall, nerves cut, mediastinum fixed

Dog	Left				Right			
	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time of oil	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time of oil
	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days
1	-4 -5	+30	+3 -3	24	-4 -6	+30	+3 -3	32
2	-3 -5	+25	+3 -3	30	-3 -5	+32	+3 -3	31
3	-4 -6	+22	+3 -3	27	-4 -6	+21	+3 -3	27
4	-4 -8	+17	+3 -4	26	-4 -7	+20	+3 -4	29
5	-3 -6	+24	+1 -3	30	-3 -6	+23	+1 -3	34

DIAPHRAGM HIGH, ABDOMINAL NERVES CUT, MEDIASTINUM FIXED

Dog	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time of oil	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time of oil
	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days
1	-2 -3	+22	+3 -2	40	-4 -6	+32	+3 -3	28
2	-1 -4	+18	+3 -2	45	-3 -6	+28	+3 -3	27
3	-2 -3	+11	+2 -3	Not out at 40 days	-4 -7	+35	+2 -3	29
4	-2 -4	+15	+2 -2	Not out at 40 days	-5 -6	+41	+2 -2	34
5	-1 -3	+15	+1 -1	Not out at 40 days	-4 -7	+24	+1 -3	32

DIAPHRAGM LOW, ABDOMINAL NERVES CUT, MEDIASTINUM FIXED

Dog	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time of oil	Intra-pleural pressure	Intra-pleural pressure during cough	Pressure in lower lobe bronchus	Emptying time of oil
	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	mm. Hg	days
1	-1 -5	+34	+3 -2	18	-3 -6	+35	-1 -6	32
2	-4 -7	+50	+2 -4	19	-2 -5	+31	-4 -8	23
3	+5 -6	+55	+1 -2	23	-1 -8	+40	-5 -6	30
4	-3 -5	+55	+2 -2	27	-3 -4	+32	-3 -6	25
5	-3 -8	+44	+1 -3	16	-4 -8	+23	-3 -3	21

**Bronchial pressure.** In the normal animal bronchial pressure varied from -1 mm. Hg during inspiration to +1 mm. Hg during expiration. During the expiratory phase of cough, the pressure rose as high as 30 to 40 mm. Hg. In normal animals with continuous irritation of the bronchus, cough was frequently sustained for 15 to 30 seconds, a result which could not be obtained, however, when the abdominal wall was weakened by removal of muscles.

In animals with fixed mediastinum, on the side with the high diaphragm there is more negative bronchial pressure during inspiration, both with cough and without; on the side with the low diaphragm, the bronchial pressure was abnormally

low during the inspiratory phase and usually higher during the expiratory phase.

### DISCUSSION

Because of the accommodation of the abdominal muscles, exact measurements of pressure and of variations in abdominal pressure are difficult to determine. From this group of experiments one factor which has been previously neglected appears to be clearly established; the abdominal pressure cannot function normally in animals with lax abdominal walls. Although abdominal pressure under such conditions does not vary greatly, its effect on thoracic function is altered or lessened, a fact which is demonstrated by a study of intrapleural and intrabronchial pressures before and after section of the abdominal muscles. This was particularly apparent following cough. Sustained coughing, which occurs frequently in normal animals with irritation of the trachea and bronchi, cannot be induced in animals with weakened abdominal walls.

We believe that the more nearly atmospheric intrapleural pressure on the side with the low diaphragm is due to distention of the lung and to diminished action of the muscles of the chest on that side. The lung is thus chronically distended and approaches the functional state of emphysema, although no anatomical changes suggesting this condition occurred. The more negative intrapleural pressure which occurred with a high diaphragm could possibly be explained on the basis of decreased activity of areas of lung with increased activity of other areas, a phenomenon similar to that observed in early lobar pneumonia. Another more likely explanation, however, has been suggested by Graham (9). He pointed out that thickening of the pleura due to adhesions and fibrosis may have been a factor in the low intrapleural pressure because of the increased resistance of the fibrous tissue to lung expansion.

Drainage of the lungs as determined by study of the time necessary to expel opaque oil readily establishes that a low diaphragm acted upon by a normal intra-abdominal pressure produces better drainage, whereas a high diaphragm with the same abdominal pressure is conducive to poor drainage of the lower lobes of the lungs. This

finding is in agreement with the observations by Graham et al. (8). He noted that in cases with basal bronchiectasis, operations to interrupt the phrenic nerve are often followed by serious consequences to the patient as a result of interference with drainage through the tracheobronchial tree. Weakened abdominal musculature leads to poor drainage of the lung regardless of the position of the diaphragm.

### SUMMARY AND CONCLUSIONS

These experiments suggest that from the standpoint of effective respiration and of pulmonary drainage there is an optimum position of the diaphragm. Since this is dependent, among other factors, upon abdominal tension, any change in pressure within the abdomen may influence pulmonary function. For optimum drainage from the lower portions of the lung a low position of the diaphragm is essential. During cough this permits a more positive expiratory bronchial pressure. The low position, however, appears to be conducive to less effective respiration, as shown by a decreased negative intrapleural pressure with normal or forced breathing. This clinically resembles the state of emphysema with low diaphragm and a diminished negative pressure.

A high position of the diaphragm appears to be conducive to fair respiratory function, as indicated by a more negative intrapleural pressure, but is less effective from the standpoint of lung drainage, as judged by the time necessary for the expulsion of opaque oil from the bronchus. It was also noted, in animals with a high diaphragm, that the expiratory bronchial pressure during cough was less than that found in normal animals. A clinical counterpart of such a condition is atelectasis of the lung. The effect of a high position of the diaphragm should also be considered in bronchiectasis and in lobar pneumonia.

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# STUDIES ON THE CIRCULATION IN PREGNANCY. I. THE VELOCITY OF BLOOD FLOW AND RELATED ASPECTS OF THE CIRCULATION IN NORMAL PREGNANT WOMEN<sup>1</sup>

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It has long been known that pregnancy imposes a "burden" upon the maternal circulation. Gain in body weight (1, 2, 3, 4), anemia (5, 6, 7), increased cardiac output (8, 9, 10, 11, 12, 13), increased oxygen consumption (8, 14, 15, 16, 17), increased blood volume (18, 19, 20), elevation in pulse rate (11, 21) and the addition of the placental circulation are all probable factors in the production of the increased load on the heart in pregnancy.

General studies on the circulation are being carried out at the Boston Lying-in Hospital in an attempt to understand the physiology of the circulation in pregnancy and to determine, if possible, the nature of this so-called "burden" on the circulation. It was hoped that such studies would lead to a practical, satisfactory method of predicting and diagnosing early heart failure in pregnant women with heart disease before the clinical signs of cardiac decompensation become evident.

This communication presents, in the main, studies on the circulation of a group of normal pregnant women, with particular emphasis on the velocity of blood flow. This aspect of the circulation in pregnancy has received little attention in contrast to its extensive study in non-pregnant individuals (22 to 37 inclusive).

The only two studies on the velocity of blood flow in pregnant women can be reviewed briefly. F. Klee (38) in 1924, studied the circulation time (which varies inversely with the velocity of blood flow), in pregnancy by the fluorescein method of Koch (37). He made single observations on 100 pregnant women in the last three months of pregnancy and found that whereas in normal non-pregnant controls the average circulation time was 20.8 seconds, in pregnant women there was a slow-

ing to 25.2 seconds in primiparae and to 23.4 seconds in multiparae. The maximum slowing occurred in the eighth month.

W. Spitzer (39) in 1933, using the decholin (sodium dehydrocholate) method of Winternitz, Deutsch and Brüll (27), studied the velocity of blood flow in 27 normal pregnant women and in 29 abnormal ones. From single observations on each patient he found no change between normal pregnant women and normal non-pregnant controls (the circulation time, arm to tongue, for normal non-pregnant individuals varied between 8 to 14 seconds, no average given; for normal pregnant ones the variation was between 10 and 16 seconds, with an average of 14.3 seconds). There was some slowing of the circulation in 4 cases of toxemia of pregnancy (25, 16.5, 26 and 25 seconds), in 3 cases of eclampsia (22, 17 and 17 seconds) and in 1 case of mitral stenosis (19 seconds). On the basis of these findings Spitzer suggested that this test might be used as an efficiency test for cardiac function in pregnant women with cardiac disease and as a test for the severity of the toxemias of pregnancy.

## PROCEDURE

The subjects for this study were obtained from the prenatal clinics of the Boston Lying-in Hospital and only those who had no serious medical or obstetrical complication were included in the normal group. Observations were made, when possible, at monthly intervals throughout pregnancy; at 2 and 6 weeks postpartum and in some patients 7 weeks or longer after delivery. The number of observations on the same patient varied between 1 and 10.

The following determinations were usually made at each visit: weight, arterial blood pressure, pulse and respiratory rates, circulation time (28) (arm to carotid, crude pulmonary and venous),

<sup>1</sup> This is the first of a series of papers concerning various aspects of the circulation in pregnancy.

vital capacity, subcostal angle, hemoglobin, hematocrit and, in some patients, venous pressure and basal metabolic rate. Seven foot x-rays of the heart and electrocardiograms were taken on some of the patients in this study. Basal metabolic rates were determined on the patients in whom there was any suspicion of thyroid dysfunction. Every patient at each visit received a careful physical examination of the heart, lungs, abdomen and extremities.

All patients were studied under basal conditions, i.e., fasting and resting. Determinations of venous pressure were preceded by at least 20 minutes rest in bed and measurements of the circulation time by at least 30 minutes rest in bed. Vital capacities were determined after the patient had been sitting upright for at least 30 minutes and with a 5 minute interval between each reading. The highest of at least 3 observations which checked within 50 cc. was taken as the value for that day. (Further details of the exact procedure used for determining the vital capacity will appear in a subsequent communication.)

#### METHODS

The arterial blood pressure was measured with a standard mercury sphygmomanometer; the venous blood pressure was determined by the direct venipuncture method of Moritz and von Tabora (40). Determinations of vital capacity were made with a simple calibrated water spirometer (Collins). Measurements of the circulation time were made according to the cyanide method of Robb and Weiss (28), which consists of the injection of sodium cyanide into a vein and measuring the time elapsing between its injection and the appearance of a characteristic respiratory response. The technique of determining the arm to carotid (referred to subsequently in this paper as the A-C), crude pulmonary (referred to subsequently as the pulmonary), and the venous circulation time, which was used throughout this study has been fully described in previous reports by Robb and Weiss (28, 29). In some instances the decholin method (which measures the arm to tongue circulation time), as modified by Gargill (34), was used in conjunction with the cyanide method.

The surface area was computed from height-

weight tables based on the formula of DuBois and DuBois (41) for surface area. The subcostal angle, except where otherwise noted, was measured with a protractor; the determining points for the angle measured being the xyphoid process, and points 7 cm. distant from it, on the border of each costal margin. Hemoglobin determinations were made with a Sahli hemoglobinometer calibrated so that 100 per cent represents 15.6 grams of hemoglobin per 100 cc. of blood. Hematocrit readings were made by the Wintrobe method (42). Edema was noted from either history or physical examination or both. Either subjective or objective evidence of shortness of breath was interpreted as dyspnea. The duration of gestation in weeks, which represents the time during pregnancy at which any given observation was made, has been calculated back from the actual date of delivery; the duration of a normal full term pregnancy being taken as 40 weeks. In those patients whose pregnancy was terminated at any point before term, the date of observation in terms of duration of pregnancy was calculated in the usual manner (Naegele's method) from the date of the last menstrual period.

#### RESULTS

Of 37 normal pregnant women studied (Table I), there are 20 primiparae, 10 secundiparae, 3 tertiparae, and 4 quadriparae. The average age of the group is 24.5 years; of the primiparae 22.9 years, of the secundiparae 24.8 years, of the tertiparae 28.3 years, of the quadriparae 28.5 years.

##### *Arm to carotid circulation time*

One hundred and forty-three determinations of the A-C circulation time were made on 36 normal pregnant women; 100 observations ante partum and 43 postpartum (Figure 1). The A-C circulation time varied between 10 and 24 seconds, ante partum, an average of 14.5 seconds; between 9 and 23 seconds, postpartum, an average of 14.9 seconds. The normal range in non-pregnant individuals by the cyanide method is from 9 to 21 seconds with an average of 15.6 seconds (28). The postpartum variation in the 7th to 81st week period was from 12 to 23 seconds, with an average time of 16.0 seconds which corresponds closely with the normal non-pregnant average of

TABLE I  
The velocity of blood flow and related aspects of the circulation in normal pregnant women

Case number	Age	Parity	Height	Weight ("normal")	Weight (observed)	Surface area	Ante partum	Postpartum	Date observed	Heart rate	Respiratory rate	Arterial pressure		Venous pressure	Vital capacity		Subcostal angle	Circulation time				Dose NaCl (A-C)		Hemo-globin		Hematocrit	Dyspnea	Edema	Remarks	
												Systolic	Diastolic		Observed	Per square meter		Arm to calf	Pulmonary	Venous	Per cent	Grams								
	2	3	4	5	6	7	8	0	10	11	12	13	14	15	cc.	Per square meter	deg.	sec. onds	sec. onds	sec. onds	21	22	23	24	25	26	27			
	10	1	07	135	160	1.75	28		Jan. 18, 1931	71	10	90	88		3800	2100	18	10	0	7	6.0	82	12.8	21	25	20	27	0	Delivered Apr. 3, 1934	
N1	21	2	00	134	132	1.00	22		Jan. 18, 1931	72	18	120	88		3150	1870	10	10	0	7	4.0	76	11.7	30.20	+	0	0	0	Delivered May 20, 1934	
N2	25	1	05	100	110	1.53	17		Jan. 18, 1931	72	18	101	70		3225	2200	15	11	3	3	5.0	73	11.4	37.10	0	0	0	0		
N3									Feb. 16, 1931	72	12	101	58		3450	2260	18	11	11	0	7.4	75	11.7	30.00	+	0	0	0	0	
N4									Mar. 11, 1931	65	16	91	61		3600	2280	85*	13	3	0	7.0	67	10.3	35.21	+	0	0	0	0	
N5									Apr. 21, 1931	60	10	88	58		3610	2200	131	14	11	0	7.0	65	10.4	31.07	+	0	0	0	0	Delivered June 30, 1934
N6									May 10, 1931	62	10	100	30		3610	2313	80	11	11	0	7.4	68	10.0	40.08	+	0	0	0	0	
N7									July 6, 1931	67	10	100	60		3630	2378	80	11	11	0	7.0	68	10.8	38.73	0	0	0	0	0	
N8									Aug 5, 1931	68	18	108	60	4.5	3620	2503	70	17	8	0	8.0	73	12.5	46.43	0	0	0	0	0	
N9	10	1	03.5	91	98	1.40	15		Jan. 10, 1931	88	18	108	70		2100	1770	11	11	3	3	4.0	00	10.8	33.00	0	0	0	0	0	Delivered May 7, 1934
N10									Feb. 23, 1931	81	10	100	63		2650	1820	14	10	3	3	5.2	00	10.8	31.20	+	0	0	0	0	
N11	23	1	02.5	100	110	1.40	20		Jan. 20, 1931	00	17	112	60		3350	2350	21	10	0	0	0.0	05	10.1	31.20	0	0	0	0	0	
N12									Feb. 10, 1931	08	20	101	08		3250	2500	16	10	0	0	0.0	03	10.3	31.10	0	0	0	0	0	Delivered Apr. 30, 1934
N13									Mar. 12, 1931	72	14	112	74		3550	2300	80*	10	0	0	0.0	08	10.0		0	0	0	0	0	
N14									Apr. 0, 1931	81	14	110	78		3010	2145	80*	19	13	7	6.4	71	11.1	56.80	0	0	0	0	0	
N15									June 15, 1934	04	10	110	80		3090	2467	85	19	13	0	0	0	56.80		0	0	0	0	0	
N16	10	1	01.5	122	113	1.51	23		Jan. 20, 1931	70	22	118	81		2000	1810	00	15	12	3	0.0	71	11.1	35.20	0	0	0	0	0	
N17									Mar. 3, 1931	72	20	112	01		2050	1016	85*	10	10	0	0.0	70	11.4	31.80	0	0	0	0	0	
N18									Apr. 1, 1931	08	10	105	06		2080	1955	00	13	10	3	0.0	68	10.0	33.02	0	0	0	0	0	
N19									Apr. 30, 1931	08	28	110	70		3120	2025	00	10	12	3	0.0	63	0.8	33.00	0	0	0	0	0	Delivered May 15, 1934
N20									May 24, 1931	08	20	124	80		3120	2025	80	11	12	3	0.4	68	10.0	56.60	0	0	0	0	0	
N21									June 22, 1931	08	24	110	60		3050	1907	65	13	13	1	0.8	73	11.7	54.08	0	0	0	0	0	
N22									June 22, 1934	04	10	100	70		2990	1943	74	18	10	5	0.0	73	12.5	40.08	0	0	0	0	0	
N23									Feb. 0, 1935	04	10	100	70	11	2860	1857	60	18	10	8	7.8	80	15.4	37.83	0	0	0	0	0	
N24	31	4	07.0	180	185	1.01	10		Jan. 22, 1931	70	22	118	74		3350	1730	100*	15	12	3	0.0	81	12.0	30.10	0	0	0	0	0	Delivered June 21, 1934
N25									Mar. 15, 1931	80	23	110	90		3250	1820	85*	10	10	3	0.4	00	10.8	33.50	+	0	0	0	0	
N26									Apr. 20, 1931	75	21	120	72		3550	1830	85*	17	11	0	0.4	00	10.8	31.40	0	0	0	0	0	
N27									May 21, 1931	81	28	118	80		3800	1000	80	14	11	0	0.4	70	10.0	31.40	0	0	0	0	0	
N28	23	1	03.5	110	110	1.50	30		Jan. 22, 1931	00	22	110	70		3300	2200	11	10	4	4	5.0	82	12.8	37.80	0	0	0	0	0	Delivered Mar. 20, 1934
N29									Feb. 19, 1931	96	12	120	88		3150	2000	12	11	1	1	5.8	70	12.3	30.23	0	0	0	0	0	
N30									Apr. 10, 1934	70	18	114	70		3400	2200	17	10	1	1	5.8	70	12.3	30.23	0	0	0	0	0	
N31									May 11, 1934	70	20	110	70		3620	2507	15	9	5	5	7.0	75	11.5	50.37	0	0	0	0	0	
N32	20	2	07.5	113	120	1.60	20		Jan. 23, 1931	08	14	100	60		3060	2100	14	11	3	3	5.0	72	11.2	32.80	0	0	0	0	0	Delivered Apr. 6, 1934
N33									Jan. 23, 1931	81	18	102	08		2000	2010	14	10	4	4	5.0	01	0.7	28.00	0	0	0	0	0	Delivered Mar. 8, 1934
N34	31	4	02.0	100	122	1.41	31		Jan. 23, 1931	80	23	110	08		3350	1991	14	10	3	3	4.4	01	0.7	28.00	0	0	0	0	0	Delivered Mar. 18, 1934
N35	25	1	07.5	115	113	1.60	31		Jan. 25, 1931	70	11	102	70		4050	2530	00*	14	11	3	0.4	70	31.70	31.70	0	0	0	0	0	
N36									Feb. 10, 1931	00	16	118	86		4000	2500	22	14	11	0	0.4	00	10.8	33.00	0	0	0	0	0	Delivered Mar. 31, 1934
N37									Mar. 10, 1931	08	14	110	00		4100	2600	00*	17	11	0	0.4	00	10.8	33.40	0	0	0	0	0	





TABLE I—Continued

Case number	Age	Parity	Height	Weight ("normal")	Weight (observed)	Surface area	Ante partum	Postpartum	Date observed	Heart rate	Respiratory rate	Arterial pressure		Venous pressure		Vital capacity		Circulation time				Hemo-globin		Hematocrit	Dyspnea	Edema	Remarks	
												Systolic	Diastolic	mm. Hg	cc.	Per square meter	Subcostal angle	Arm to carotid	Pulmonary	Venous	Per cent	Grams						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
	10	1	03.0	150	143	1.71	20	2	Feb. 8, 1931	81	15	108	88		3800	2250			15			0.4	70	11.0	31.00	+	0	Delivered June 28, 1934
	27	2	01.0	105	122	1.50	38	2	July 12, 1934	64	10	100	70		4050	3383			15			0.0	70	11.4	30.60	+	0	
	28	2	01.0	105	122	1.50	38	2	Feb. 10, 1931	72	16	123	78		3300	2200		70*	10			0.4	80	12.5	35.00	0	0	Delivered Feb. 25, 1934
	25	3	02.0	100	117	1.44	21	17	June 26, 1934	68	10	110	80		3050	2030		70	17			0.4	81	12.5	35.50	0	0	
	20	3	02.0	100	117	1.44	21		Feb. 13, 1931	90	20	102	68		3325	2300			11	11	3	5.0	82	12.8	30.10	0	0	Delivered June 28, 1934
	35	1	00.0	131	130	1.00	17		Feb. 10, 1931	81	16	136	82		3700	2100		00*	12	10	3	0.0	93	13.7		0	0	Delivered July 25, 1934. The baby weighed 6 pounds 8 ounces at birth, 18 hours after delivery
	30	32		140	140		30		May 28, 1931	88	20	130	80		3500	2108		85	13	10	3	7.0	71	11.5		+	0	pounds 8 ounces at birth, 18 hours after delivery
	30	32		140	140		30		June 20, 1931	85	13	112	64		3000	2131		85	13	1	0.4	70	11.0	30.83	+	0	Delivered Feb. 25, 1934	
	30	32		140	140		30		Aug. 5, 1934	84	20	150	68		3550	2116		00	13	11	3	0.8	87	13.0	40.40	0	0	Delivered June 28, 1934
	25	1	02.0	105	108	1.40	14	14	Feb. 10, 1931	73	10	102	68		2300	1670		80*	15	10	3	0.0	72	11.0	35.22	0	0	Delivered July 25, 1934. 7 foot x-ray photo of heart and electrocardiogram normal. Basal metabolic rate on July 10, 1931, +12 per cent; on Nov. 11, 1935, minus 2 per cent
	25	1	02.0	105	108	1.40	14	14	Mar. 12, 1931	73	10	100	68		2250	1670		80	15	10	3	0.4	72	11.0	35.22	0	0	
	25	1	02.0	105	108	1.40	14	14	Apr. 27, 1931	68	12	101	60		2150	1470		80	15	10	3	0.0	84	0.6	33.30	+	0	
	25	1	02.0	105	108	1.40	14	14	May 21, 1931	80	18	100	74		2150	1700		80	15	10	3	0.0	81	0.6	33.30	+	0	
	25	1	02.0	105	108	1.40	14	14	June 18, 1931	68	10	120	80		2520	1730		00	15	10	3	0.0	80	10.8	33.83	+	0	
	25	1	02.0	105	108	1.40	14	14	July 10, 1931	68	10	120	80		2570	1702		02	15	10	3	0.0	80	8.7	36.80	+	0	
	25	1	02.0	105	108	1.40	14	14	Aug. 27, 1934	64	20	108	78		2640	1719		05	13	11	3	0.4	70	11.8	36.80	+	0	
	25	1	02.0	105	108	1.40	14	14	Sept. 24, 1934	68	20	102	65		2650	1719		07	13	11	3	7.0	73	11.8	36.80	+	0	
	25	1	02.0	105	108	1.40	14	14	Oct. 1, 1935	73	10	100	70		2530	1735		04	13	11	7	7.4	71.7	40.07	0	0		
	25	1	02.0	105	108	1.40	14	14	Nov. 14, 1935	73	18	100	60		2460	1655		05	13	9	6	6.8	70	11.8	36.60	0	0	
	20	1	03.0	122	106	1.58	32	32	Feb. 17, 1931	80	11	110	71		2850	1800		90*	17	11	0	0.0	77	12.0		0	0	Delivered Apr. 13, 1934
	32	1	01.5	121	125	1.55	12	12	Feb. 27, 1931	60	28	115	72		3125	2016		85*	16	12	4	0.0	70	10.0	30.10	+	0	Delivered Sept. 13, 1934
	20	1	00.0	135	133	1.58	20	20	May 25, 1931	81	24	110	60		2000	1700		85	14			0.0	62	0.7	32.00	0	0	Delivered Oct. 12, 1934
	24	2	01.0	110	126	1.50	21	21	Sept. 11, 1931	80	11	120	70		3770	2117		90	13	11	2	0.0	98	10.6	33.08	+	0	
	24	2	01.0	110	126	1.50	21	21	Oct. 11, 1931	81	18	110	68		3720	2351		11	10	11	0	0.0	75	11.7	31.48	+	0	
	24	2	01.0	110	126	1.50	21	21	Nov. 8, 1931	81	18	120	70		4.3	3520		70	11	10	4	0.4	68	10.6	30.02	+	0	
	24	2	01.0	110	126	1.50	21	21	Dec. 6, 1931	81	22	114	70		3000	2308		72	11	9	5	7.3	70	10.0	30.02	+	0	
	24	2	01.0	110	126	1.50	21	21	Jan. 4, 1935	80	11	110	60		3080	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Feb. 2, 1935	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Mar. 18, 1935	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Apr. 7, 1935	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	May 7, 1935	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	June 7, 1935	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	July 7, 1935	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Aug. 7, 1935	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Sept. 7, 1935	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Oct. 7, 1935	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Nov. 7, 1935	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Dec. 7, 1935	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Jan. 7, 1936	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Feb. 7, 1936	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Mar. 7, 1936	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Apr. 7, 1936	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	May 7, 1936	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	June 7, 1936	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	July 7, 1936	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Aug. 7, 1936	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Sept. 7, 1936	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	
	24	2	01.0	110	126	1.50	21	21	Oct. 7, 1936	78	10	150	80		3050	2308		95	11	9	5	7.2	67	10.6	32.33	+	0	

TABLE I—Continued

No.	Sex	Age	Height (cm.)	Weight (kg.)	Weight (lb.)	Surface area (sq. m.)	Ante partum (wks)	Parturition	Date observed	Heart rate (per minute)	Respiratory rate (per minute)	Arterial pressure		Venous pressure (cm. H <sub>2</sub> O)	Vital capacity		Subcostal angle	Circulation time			Dose NaCl (A-C) (mgm.)	Hemo-globin		Hematocrit (per cent)	Dyspnea	Edema	Remarks	
												Systolic (mm. Hg)	Diastolic (mm. Hg)		Observed (cc.)	Per square meter (cc.)		Arm to carotid (sec-onds)	Pulmonary (sec-onds)	Venous (sec-onds)		Per cent	Grams					
N-3	F	23	162	102	225	1.44	16	9	10	88	16	124	70	7.8	2700	1875	45	13	0	4	0.6	73	11.4	36.40	+	0	0	Delivered June 8, 1935. 7 foot x-ray plate of heart and electrocardiogram normal. Basal metabolic rate on Nov. 9, 1935, was minus 17 per cent
												110	60	8.0	2720	1890	50	11	9	2	8.4	69	10.8	31.80				
												112	60	8.0	2720	1890	70	11	0	2	0.5	72	11.2	34.41				
												117	78	8.0	2730	1894	70	13	8	5	6.7	67	10.5	34.02				
												121	60	8.5	2780	1931	75	12	10	2	6.7	66	9.4	29.08				
												125	80	9.5	2700	1937	75	11	10	1	6.8	66	10.3	32.64				
												112	64	12.0	2680	1861	65	10	9	1	7.0	69	9.3	31.91				
												104	70	10.6	2680	1861	65	10	9	1	7.4	68	10.6	36.60				
												109	70	9.4	2660	1840	65	14	9	5	7.3	84	13.1	40.88				
												100	50	8.8	3510	2296	60	17	12	5	5.8	73	11.4	35.88				
N-4	F	23	160	119	153	1.53	14	18	10	78	20	96	58	6.1	3500	2288	60	19	12	5	5.8	76	11.9	35.00	+	0	0	Delivered Aug. 27, 1935. 7 foot x-ray plate of heart and electrocardiogram normal
												100	56	6.2	3610	2360	70	19	12	6	5.8	76	11.9	35.00				
												100	56	6.2	3610	2360	70	19	12	6	5.8	76	11.9	35.00				
												100	56	8.5	3400	2248	72	19	12	6	5.8	69	10.8	31.82				
												100	50	8.5	3440	2248	72	19	12	6	5.8	68	10.6	32.88				
												100	50	10.0	3620	2365	90	19	12	6	5.8	65	10.1	31.82				
												100	50	10.0	3490	2260	90	19	12	6	5.8	65	10.1	31.82				
												100	70	6.0	3780	2361	60	15	11	4	4.2	73	11.4	41.17				
												110	70	6.7	3620	2291	65	16	12	4	4.4	75	11.7	37.92				
												115	70	6.1	3800	2404	70	15	12	3	4.1	69	10.7	34.72				
N-5	F	24	161	110	1.58	13	17	12	10	80	24	110	70	6.0	3780	2361	60	15	11	4	4.2	73	11.4	41.17	0	0	0	Delivered Sept. 22, 1935. 7 foot x-ray plate of heart and electrocardiogram normal
												115	70	6.7	3620	2291	65	16	12	4	4.4	75	11.7	37.92				
												110	60	6.1	3800	2404	70	15	12	3	4.1	69	10.7	34.72				
												110	60	7.0	3610	2303	80	13	11	2	4.8	72	11.2	34.72				
												104	60	6.9	3510	2222	100	12	11	2	5.4	80	0.4	32.88				
												120	80	6.8	3480	2202	104	12	10	2	5.4	83	10.6	34.08				
												116	78	6.7	3560	2262	65	14	9	5	6.6	80	12.5	41.36				
												110	70	6.0	2800	1920	70	18	11	7	5.6	60	0.4	30.24				
												98	70	2870	1966	80	15	10	5	6.0	53	8.3	28.42					
												100	60	2920	1997	85	13	9	4	6.0	51	8.0	27.20					
N-6	F	22	160	118	1.52	29	18	37	10	84	18	114	78	8.0	2860	1860	65	16	11	4	6.2	64	10.0	28.50	0	0	0	Delivered Apr. 12, 1934. 7 foot x-ray plate of heart normal
												114	86	7.1	2900	1910	75	16	11	4	6.2	64	10.0	28.50				
												108	68	7.1	2900	1910	75	16	11	4	6.2	64	10.0	28.50				
												114	70	7.5	2940	1930	75	16	11	4	6.2	64	10.0	28.50				
												114	70	7.5	2940	1930	75	16	11	4	6.2	64	10.0	28.50				
												114	70	7.5	2940	1930	75	16	11	4	6.2	64	10.0	28.50				
												114	70	7.5	2940	1930	75	16	11	4	6.2	64	10.0	28.50				
												114	70	7.5	2940	1930	75	16	11	4	6.2	64	10.0	28.50				
												114	70	7.5	2940	1930	75	16	11	4	6.2	64	10.0	28.50				
												114	70	7.5	2940	1930	75	16	11	4	6.2	64	10.0	28.50				

\* Estimated.  
† Measured in cm. of normal saline, reported in cm. of H<sub>2</sub>O.

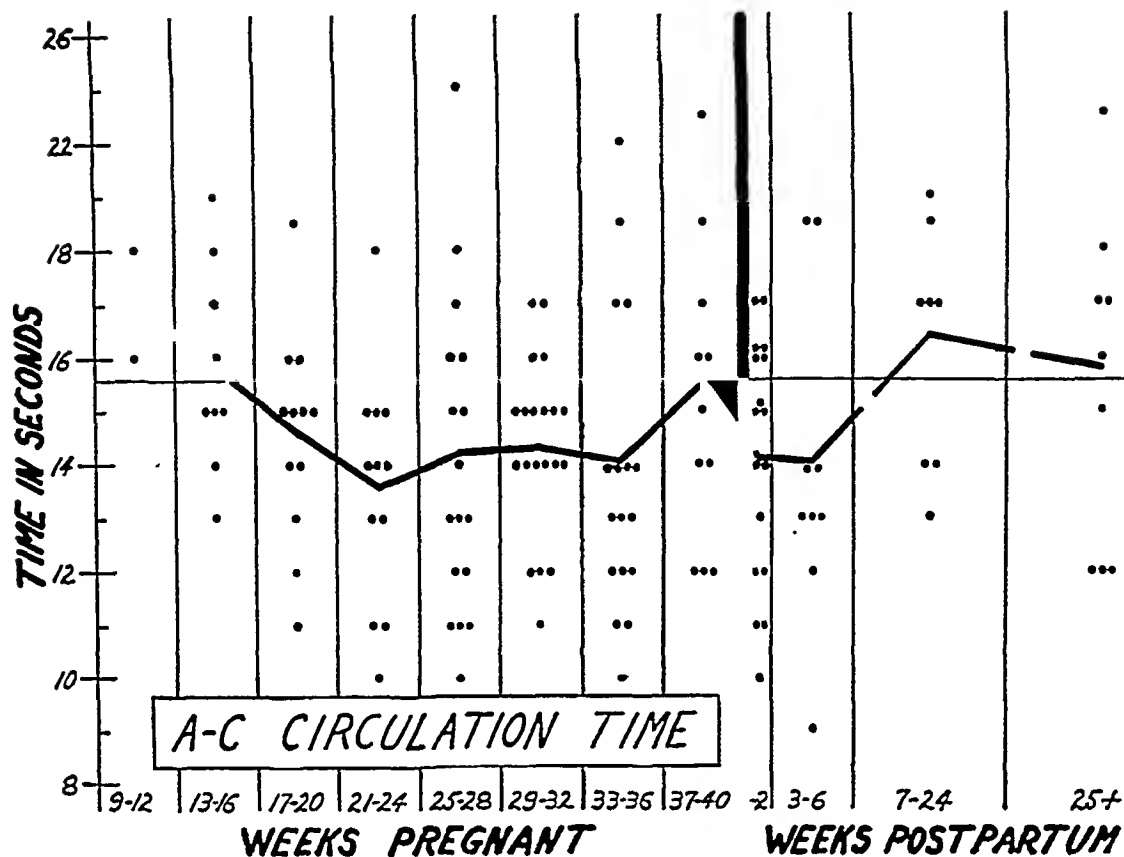


FIG. 1. THE ARM TO CAROTID CIRCULATION TIME IN NORMAL PREGNANT WOMEN.

The solid dots represent individual observations; the solid heavy line represents the average values. The horizontal black line represents the average normal non-pregnant A-C circulation time. The heavy perpendicular line represents delivery and separates the ante and postpartum periods.

15.6 seconds. These late postpartum observations constitute a normal control group for this study.

The shortening of the circulation time indicated by the curve of average values (Table II) did not

occur invariably or at every observation, although present in the majority of repeatedly studied cases. In a few instances there was prolongation, while occasionally no change could be detected.

TABLE II

The average A-C, pulmonary and venous circulation times; the average pulse rate, hemoglobin, hematocrit and viscosity of the blood at varying intervals during pregnancy and the puerperium

	Weeks pregnant								Weeks postpartum			
	9 to 12	13 to 16	17 to 20	21 to 24	25 to 28	29 to 32	33 to 36	37 to 40	1 to 2	3 to 6	7 to 24	25 +
A-C circulation time, seconds	17.0	15.9	14.6	13.6	14.2	14.3	14.0	15.5	14.1	14.0	16.4	15.8
Pulmonary circulation time, seconds	11.6	10.7	10.4	10.4	9.7	10.0	10.0	10.6	10.7	10.1	10.6	9.8
Venous circulation time, seconds	5.3	5.2	3.8	3.4	4.3	4.2	3.0	5.4	3.6	3.6	5.2	6.5
Pulse rate	84.0	79.8	81.8	77.4	82.1	79.8	81.6	80.0	69.3	69.7	70.0	71.0
Hemoglobin, per cent	73.3	72.0	72.4	67.3	67.8	67.1	64.6	67.7	69.6	69.5	74.8	78.4
Hematocrit, per cent	37.83	35.49	34.21	33.95	33.47	32.93	32.90	35.08	36.64	37.50	39.28	39.03
Viscosity, relative to water	4.685	4.456	4.331	4.305	4.258	4.205	4.202	4.416	4.569	4.753	4.827	4.893

The ante and postpartum values fell within the normal non-pregnant range in all but 4 instances; 3 ante partum and 1 postpartum (Table I, Cases N7, N16, N17 and N25). In contrast to Klee's findings (38) the average circulation time for primiparae and multiparae in this group was essentially the same; for primiparae the average ante partum A-C time was 14.6 seconds; for multiparae 14.4 seconds.

No obvious correlation existed between the A-C circulation time and pulse rate or hemoglobin content of the blood (Figures 2 and 3).

#### *Pulmonary circulation time*

One hundred and twelve determinations of the pulmonary circulation time were made on 34 normal pregnant women; 78 before and 34 after de-

livery (Figure 4). The pulmonary circulation time varied between 9 and 13 seconds ante partum, an average of 10.7 seconds; the postpartum variation was from 8 to 14 seconds, an average of 10.3 seconds. The variation in the pulmonary circulation time by the cyanide method in normal non-pregnant individuals is from 7 to 14 seconds with an average of 10.6 seconds (28). All of the values in the normal pregnant group are within the normal non-pregnant range and the average of 10.7 seconds for all the ante partum observations corresponds closely with the average of the normal non-pregnant individuals of 10.6 seconds; the average for all the postpartum values is 10.3 seconds.

Here again there is a definite slight shortening of the average pulmonary circulation time during

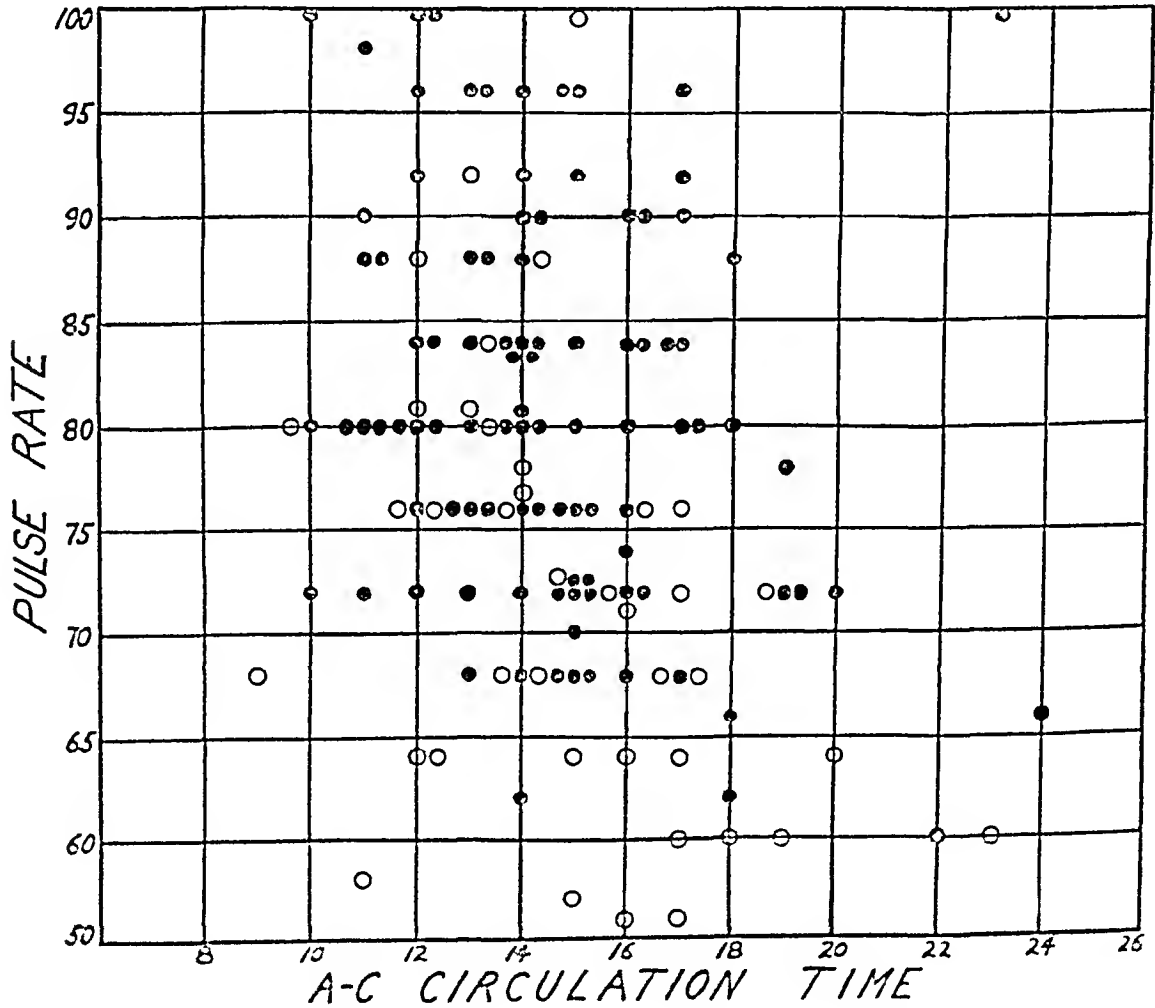


FIG. 2. THE RELATIONSHIP BETWEEN THE PULSE RATE AND THE ARM TO CAROTID CIRCULATION TIME.

The solid dots represent individual ante partum observations, the circles represent individual postpartum observations.

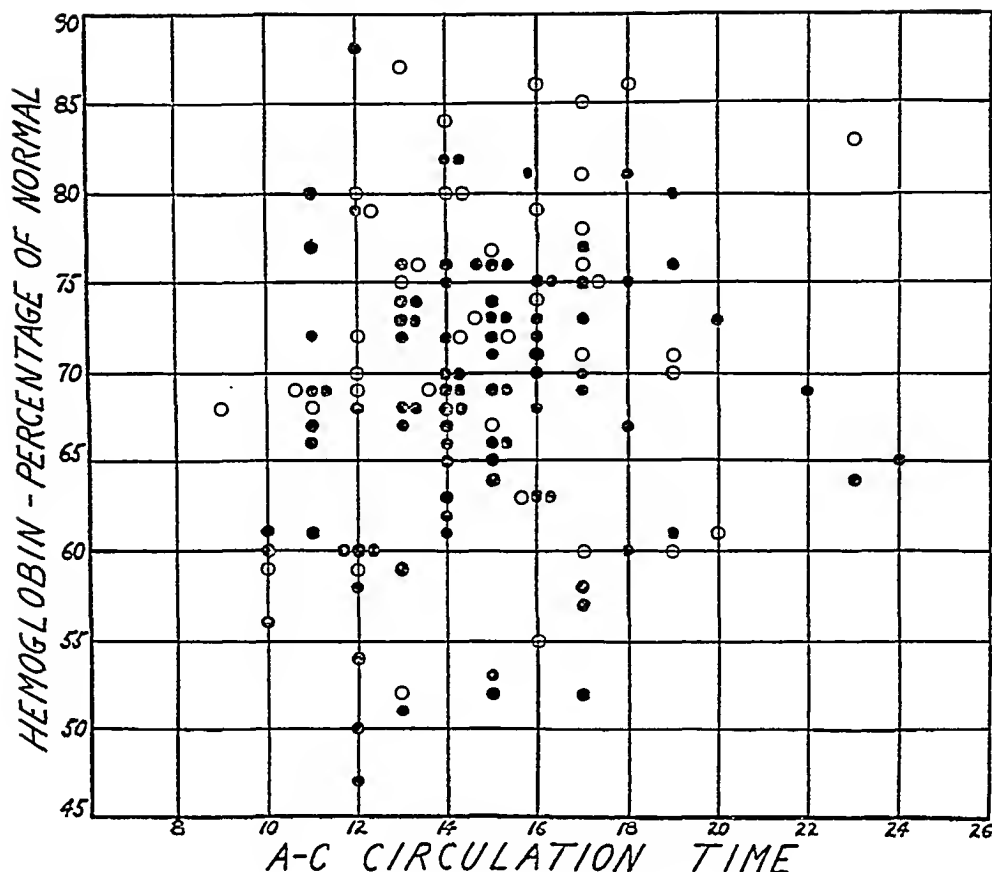


FIG. 3. THE RELATIONSHIP BETWEEN THE HEMOGLOBIN AND THE ARM TO CAROTID CIRCULATION TIME.

The solid dots represent individual ante partum observations, the circles represent individual postpartum observations.

pregnancy (Table II) although the change could not be detected in every case.

There was no obvious correlation between the pulmonary circulation time and the pulse rate and hemoglobin respectively (Figures 5 and 6).

#### *Venous circulation time*

The venous circulation time was calculated 108 times, 76 before, 32 after delivery, in 29 normal pregnant women. It varied between 1 and 9 seconds ante partum (average 4.1 seconds). The postpartum variation was between 1 and 10 seconds (average 4.5 seconds). The average venous time after the seventh week postpartum was 6.0 seconds. The normal non-pregnant variation in venous time by the cyanide method is from 1 to 9 seconds (average 4.5 seconds) (28). Since the

average pulmonary circulation time showed relatively slight change throughout pregnancy and the puerperium (Figure 4) and because of the method of calculating the venous time, it is evident that the changes in the average venous time will vary as the A-C circulation time varies (Figure 7).

No correlation between either the pulse rate or hemoglobin and the venous circulation time could be demonstrated.

#### *Dose of sodium cyanide*

The effective dose of sodium cyanide for measuring the A-C circulation time varied between 4.0 and 9.0 mgm. per patient (corresponding to 0.2 cc. to 0.45 cc. of a 2 per cent aqueous solution of

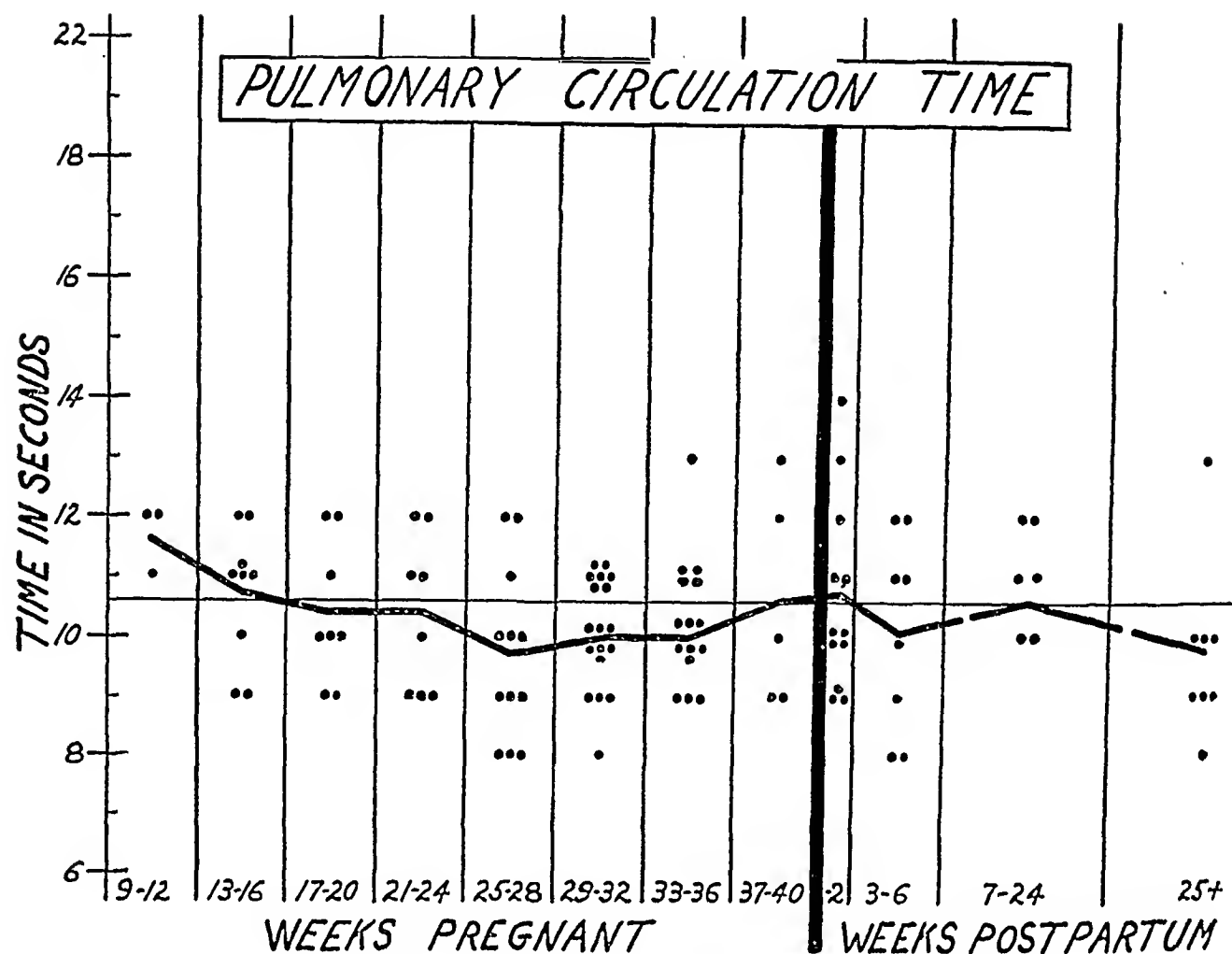


FIG. 4. THE PULMONARY CIRCULATION TIME IN NORMAL PREGNANT WOMEN.

The solid dots represent individual observations; the solid, heavy line represents the average values. The horizontal black line represents the average normal non-pregnant pulmonary circulation time. The heavy perpendicular line represents delivery and separates the ante and postpartum periods.

NaCN). The dose per kilogram of body weight ante partum varied between 0.067 and 0.142 mgm. (average 0.102 mgm.). The average postpartum dose per kilogram of body weight was 0.127 mgm.; the smallest effective dose postpartum was 0.092 mgm. per kilo; the largest was 0.170 mgm. per kilo. The effective dose of sodium cyanide for non-pregnant normal individuals ranged from 5.0 to 10.0 mgm., or from 0.07 to 0.19 mgm. per kilogram of body weight, with an average dose of 7.0 mgm. or 0.11 mgm. per kilogram body weight (28). In 2 patients in this series the dose per kilogram body weight was less postpartum than ante partum; in 14 it was more and in 3 it remained the same. The effective dose of sodium cyanide required in determining the pulmonary circulation time was less in all instances than that

required for determining the A-C time, averaging about 75 per cent of the effective A-C dose. This is essentially what is found in non-pregnant normal subjects (28).

#### *Venous pressure*

Determinations of venous pressure were made on 10 patients, but in only 5 were they made ante partum (Cases N36, N37, N38, N39 and N40, Table I).

It varied between 4.0 and 12.0 cm. of water ante partum and between 6.4 and 12.0 cm. postpartum. The average ante partum was 7.9 cm. of water, and postpartum 9.1 cm. All of the values were within normal limits (12.0 cm. or less). In the one patient in whom it was consistently above 10 cm. it should be noted that rather marked

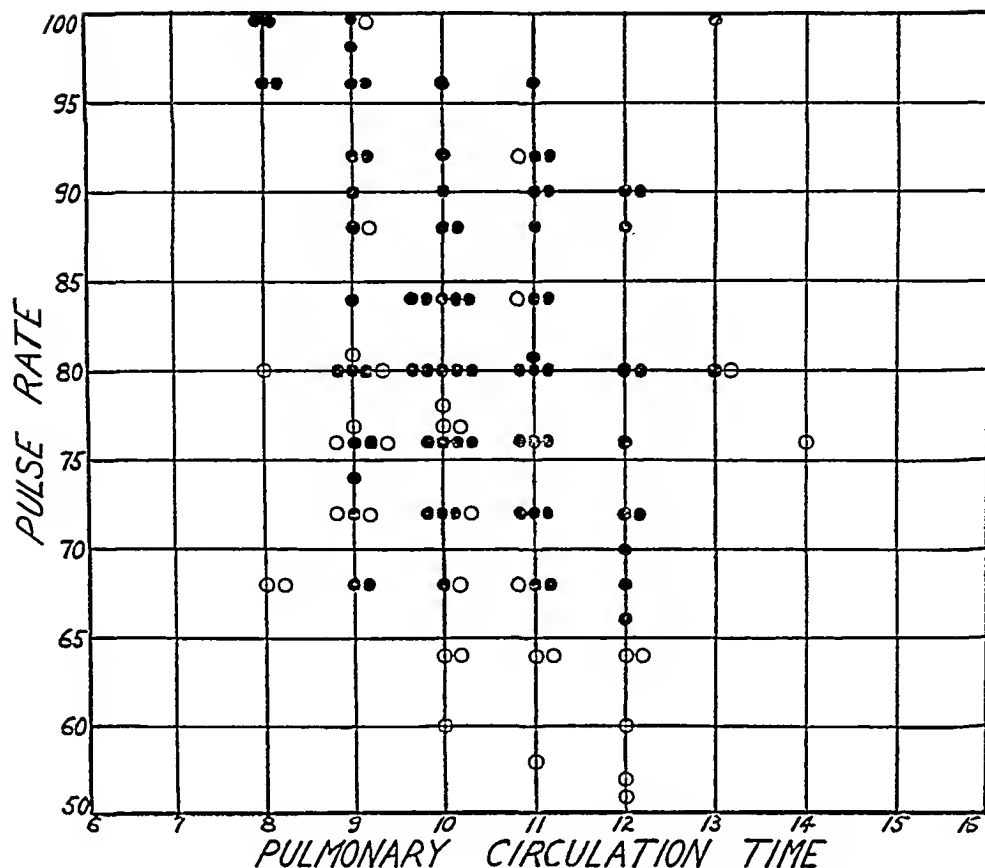


FIG. 5. THE RELATIONSHIP BETWEEN THE PULSE RATE AND THE PULMONARY CIRCULATION TIME.

The solid dots represent individual ante partum observations, the circles represent individual postpartum observations.

obesity was present, since it is high in obesity (43, 44). In Cases N36 and N38 the postpartum values were higher than the ante partum values.<sup>2</sup>

#### *Vital capacity*

One hundred and fifty-two determinations of the vital capacity were made on 37 normal pregnant women; 108 before and 44 after delivery. The values in most instances were within the limits set as normal for non-pregnant women, i.e., 2000 cc. per square meter body surface area (45, 46). Thirty-four of the 37 had vital capacities of at least 90 per cent of normal and 36 at least 85 per

cent of normal, only one (Case N31) falling below 85 per cent. In this patient it was 74 per cent of normal at its lowest level in the 24th week and 88 per cent at its highest in the 36th week. During postpartum observations on this patient, made at 2, 6, 26 and 65 weeks after delivery, the vital capacity never exceeded its highest pregnancy level of 88 per cent of normal. It is sufficient to note here<sup>3</sup> that the vital capacity either remained constant or rose during the course of pregnancy in the majority of the patients in this group; in a few there was a slight decrease as pregnancy progressed.

<sup>2</sup>Further observations on the venous pressure in pregnancy will appear in a subsequent communication.

<sup>3</sup>Further discussion of the observations on the vital capacity in pregnancy will appear in a subsequent paper.



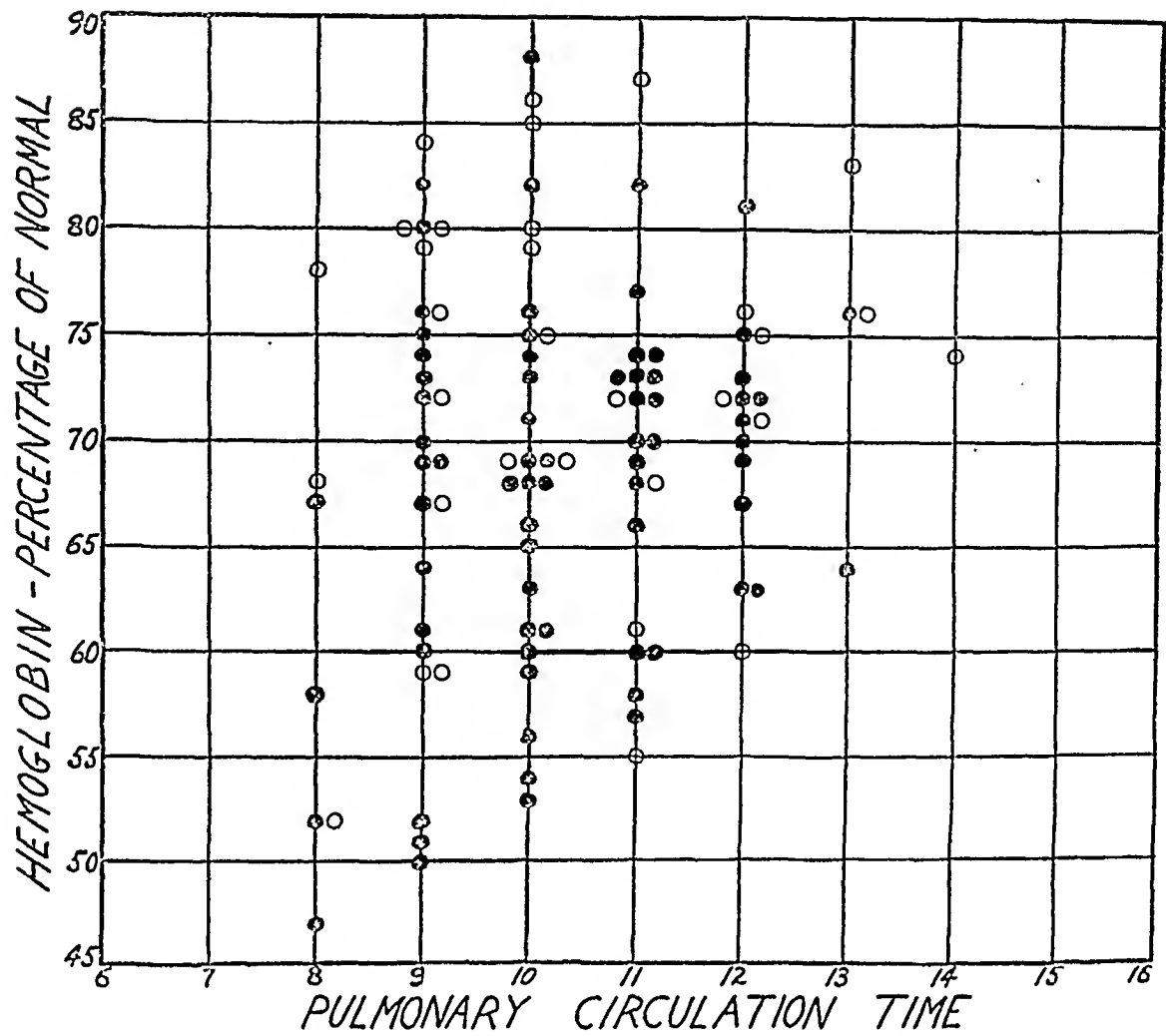


FIG. 6. THE RELATIONSHIP BETWEEN THE HEMOGLOBIN AND THE PULMONARY CIRCULATION TIME.

The solid dots represent individual ante partum observations, the circles represent individual postpartum observations.

*Subcostal angle*

In all but 2 patients (Cases N25 and N30), in whom the subcostal angle was measured with a protractor, there was an increase as pregnancy progressed with a decrease after delivery (Table I). The maximum increase observed during pregnancy was 44°.

*Blood pressure*

The basal arterial blood pressure (Table I) was within the limits set as normal for pregnant women (47) except for 2 cases (Case N20 at 2 weeks postpartum and Case N16 at 38 weeks pregnant).

*Pulse rate*

The pulse rate ante partum (Table I) under basal conditions varied from 60 to 100 beats per

minute (average 80.6 beats). Postpartum, it varied between 52 and 92 (average 70 beats).

*Respiratory rate*

The basal respiratory rate (Table I), ante partum, varied between 10 and 28 per minute (average 18.0 per minute). Postpartum, the variation was between 10 and 28 (average 17.5).

*Blood*

Hemoglobin determinations and hematocrit readings were carried out at frequent intervals before and after delivery (Table I). In every subject observed two or more times during gestation there was a fall in the hemoglobin, as pregnancy progressed. This change was usually accompanied by a fall in the hematocrit reading.

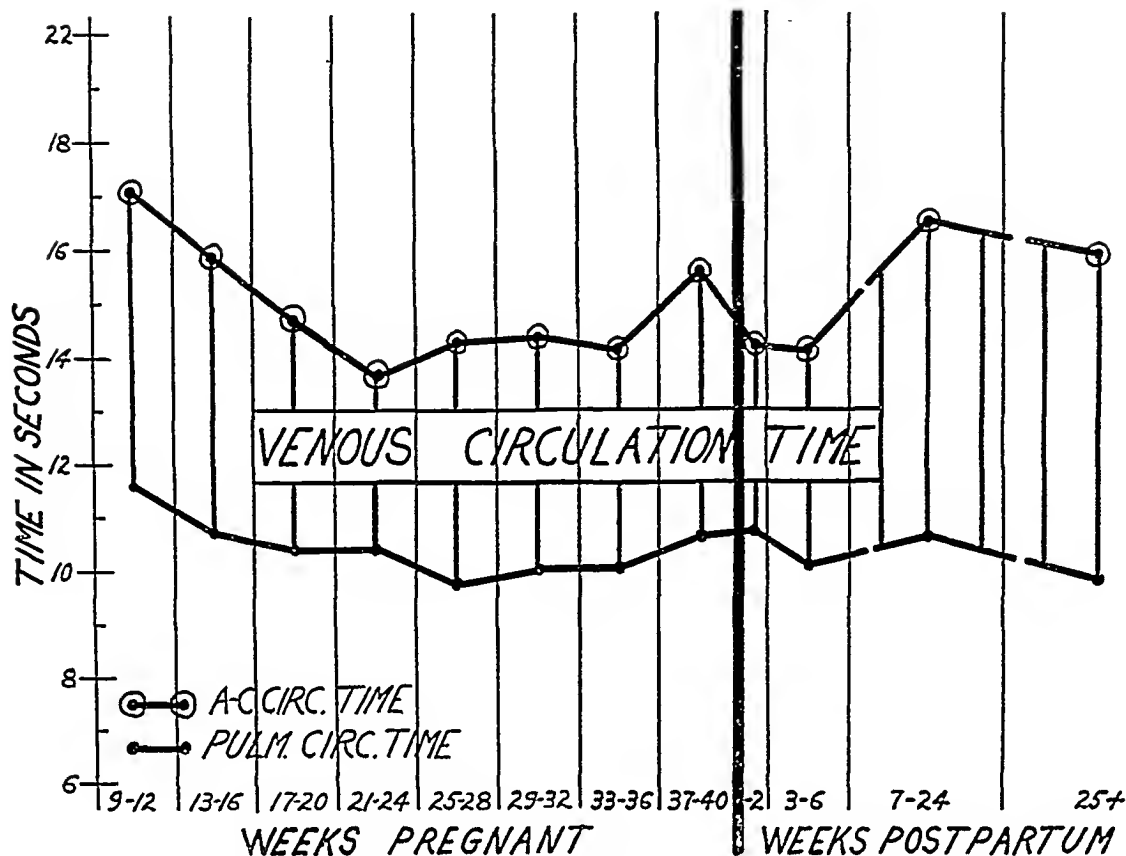


FIG. 7. THE VENOUS CIRCULATION TIME IN NORMAL PREGNANT WOMEN.

The upper curve represents the average arm to carotid circulation time; the lower curve represents the average pulmonary circulation time; the shaded area between represents the average venous circulation time. The heavy perpendicular line represents delivery and separates the ante and postpartum periods.

During the latter weeks there was a tendency for both measurements to rise (Figure 8). This observation is in accord with that of Kühnel (5). Of 15 patients, 10 showed an increase in the postpartum hemoglobin value; 5 showed a decrease. Of 18 patients, the hemoglobin rose in 14 by the 7th week postpartum; 4 showed a decrease.

#### DISCUSSION

##### Method

The cyanide method proved to be practical for studying the velocity of blood flow in pregnant women. Sodium cyanide, in the doses reported here, can be administered to pregnant women without harm to either mother or child. Early in the study, the fetal heart was examined with the fetoscope during and after the administration of

cyanide and no apparent change in rate or rhythm was noted. No ill effects to the baby were demonstrable at birth or afterwards. There was one fetal death but it was clearly unrelated to the administration of cyanide. (Table I, Case 30. The last dose of cyanide was given one month before delivery. The baby died 18 hours after delivery of respiratory failure. The autopsy showed intracranial hemorrhage and pulmonary atelectasis.)

An occasional patient, usually one to whom a relatively large dose of cyanide was given, showed, after the respiratory response, flickering of the eyelids. Rarely, coincident with this, there was a period during which the patient could not speak for a few moments but could comprehend and execute physical commands. Such reactions were of short duration and did not prevent further tests. Antidotes against cyanide, namely, amyl

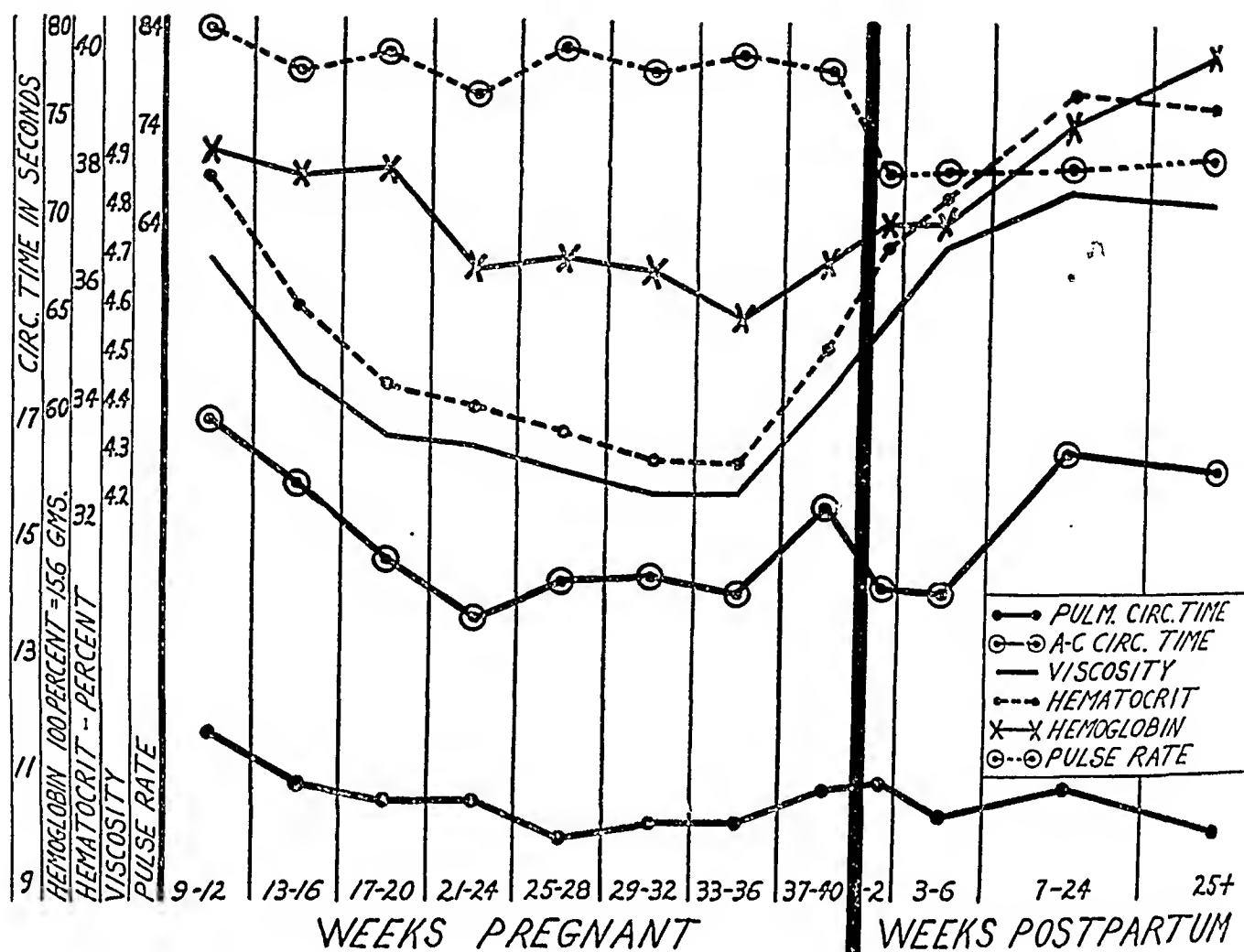


FIG. 8. THE RELATIONSHIP BETWEEN THE AVERAGE ARM TO CAROTID AND PULMONARY CIRCULATION TIMES AND THE AVERAGE VISCOSITY OF THE BLOOD, THE AVERAGE HEMATOCRIT, THE AVERAGE HEMOGLOBIN AND THE AVERAGE PULSE RATE IN 37 NORMAL PREGNANT WOMEN.

The heavy perpendicular line represents delivery and separates the ante and postpartum periods. For numerical values see Table II.

nitrite, sodium nitrite, sodium thiosulphate and methylene blue (48), were always at hand but it was never necessary to use them.

The decholin method of Winternitz, Deutsch and Brüll (27), as modified by Gargill (34), was used in a few patients. Because, in some there was vomiting and in others the response was not definite or consistent, perhaps due to the raising, by 60 per cent, of the threshold for bitter taste in pregnancy described by Hansen and Langer (49), this method was not felt to be as satisfactory as the cyanide method.

For pregnant women the effective dose of sodium cyanide was less per kilogram of body weight than that used in normal non-pregnant subjects (28). The increase in the weight of the

mother caused by pregnancy might cause a relative decrease in dosage; but this explanation is probably incorrect because the dose required is usually greater after delivery than before. The smaller dose might reflect an increased sensitivity of the carotid sinus in pregnancy.

#### Results

The values for the A-C circulation time in normal pregnancy fall almost without exception within the normal non-pregnant limits of 9 to 21 seconds (28). From the 17th, through the 36th week of gestation, there seems to be a definite decrease in the average A-C circulation time. The diminished arteriovenous oxygen difference in pregnancy, and the lag in oxygen consumption

as compared with the cardiac output, demonstrated by Burwell and Strayhorn (8), are additional evidence that the circulation is speeded during pregnancy.

In the last 4 weeks (37th to 40th) there is an apparent increase in the A-C circulation time as compared with the preceding weeks. The number of observations in the 37th to 40th week period presented here is not sufficient to establish this point. The fact that the pulmonary circulation shows a parallel, though slight, rise during the same period, and the observation that a group of 33 pregnant women with compensated heart disease (50) shows a similar change in the last 4 weeks of pregnancy, are, however, further corroborative evidence. Burwell and Strayhorn (8) have shown, furthermore, that the cardiac output increases during pregnancy but that during the last weeks it is less than in the preceding months. Gammeltoft (10, 11) found a similar change. This change is corroborative evidence that the A-C circulation time is increased in the 37th to 40th week period, if the assumption is correct that, other factors remaining equal, the speed of the circulation varies directly with the cardiac output (51).

Following delivery there is again a decrease of the A-C circulation time, which seems to persist for several weeks after delivery (Figure 1). Further evidence that the circulation is speeded immediately postpartum and for some time afterwards, is given by Gammeltoft (10, 11) and Haupt (13), who showed that the cardiac output, although less postpartum than ante partum, did not return to the normal non-pregnant level for several weeks (as long as 4 months in 1 patient of Gammeltoft) after parturition. From the observations presented here, it appears that the A-C circulation time returns to normal certainly by the sixth month postpartum and probably earlier.

The explanation of the decrease in the circulation time during pregnancy and early in the puerperium (to the 7th week) is not clear. The various factors known to affect the velocity of blood flow in non-pregnant individuals should be considered.

Anemia in non-pregnant individuals has been reported to cause a decrease in the pulmonary, arm to arm, and arm to tongue circulation times

(22, 30, 34) and histamine reaction time (35). In most of the patients in this series a fall in hemoglobin and hematocrit values typical of pregnancy was observed (5). There seems to be no apparent correlation, however, between the per cent of hemoglobin and the A-C circulation time when these two factors are plotted against each other (Figure 3). Neither is there any apparent correlation between the individual hematocrit readings and A-C circulation time. Yet, when the average values for hemoglobin and hematocrit readings are plotted, the resultant curves are similar to the course of the average A-C circulation time during pregnancy (Figure 8). Immediately after delivery and up to the 7th week postpartum this apparent similarity is absent.

A decrease in the pulmonary, arm to arm, and arm to tongue circulation times (22, 25, 34, 36) and histamine reaction time (35) has been reported in non-pregnant cases of *hyperthyroidism*. It has also been reported that the basal metabolic rate is increased in pregnancy (15, 52, 53, 54). This might account for the decreased circulation time demonstrated in this series. The occasional measurements of basal metabolic rate, while not sufficient in number to be conclusive, were within normal limits, however, in spite of the decreased circulation time. The postpartum decrease in the A-C circulation time observed is probably not due to increase in the basal metabolic rate, since this and oxygen consumption are reported to return to normal within a few days postpartum (15, 16, 53), while the cardiac output and velocity of blood flow do not for several weeks at least (10, 11, 13).

The *pulse rate* is elevated during pregnancy and drops on the average about 10 beats per minute (Table II) after delivery. There seems to be no correlation, however, between the A-C circulation time and pulse rate as regards either individual observations (Figure 2) or average values (Figure 8).

There is an increased *cardiac output* during pregnancy (8, 9, 10, 11, 12, 13) which might contribute to the decrease in the A-C circulation time or might be dependent upon it. Further study is necessary to elucidate this point.

It was shown by Esiaschwili (55) that the *viscosity of the blood* is decreased in pregnancy

up to the sixth month, is slightly increased in the seventh month, is unknown in the latter months, and presents a consistent postpartum rise. It is known that the speed of flow of a liquid varies inversely with its viscosity (Poiseuille's law). Lowered blood viscosity would result accordingly in increased velocity of blood flow.

Nygaard, Wilder and Berkson (56) showed that the relation of the viscosity of whole blood to the hematocrit readings may be expressed by the linear equation  $V_{wb} = 0.978 + 0.098H$ , where  $V_{wb}$  = the viscosity of whole blood and  $H$  = hematocrit reading in per cent (this relationship holds only when the hematocrit reading ranges between 15 to 50 per cent).

The average viscosity was calculated, from this equation, for the various periods of pregnancy and the puerperium. There is a steady decrease to the 37 to 40th week period (Table II) when an increase occurs and continues on through the postpartum period.

When the average viscosity values are plotted with the average values of the A-C circulation time there is a similarity in the curves (Figure 8) which suggests that there is a definite relationship between the blood viscosity and the velocity of blood flow. Immediately after delivery, however, and up to the 7th week postpartum this apparent similarity is lacking. Although the complex bodily readjustments which occur during this time (from delivery to the 7th postpartum week), make a simple explanation of the speeding of the A-C circulation difficult, it seems possible that lactation, with its concomitant physiological changes, is an important factor.

From the preceding discussion it is evident that there are several factors which might produce the acceleration of the circulation in pregnancy. The decrease in the viscosity of the circulating blood would seem to be one of the most important since the changes in the velocity of blood flow which occur simultaneously with, or are dependent upon it, coincide with Poiseuille's law.

The results presented here seem at first glance to disagree with the work of Klee (38) who found a slowing in the rate of flow of the circulation in pregnancy. However, the fluorescein method of Koch (37) which he used, necessitates that the test substance traverse the peripheral capillary circulation, which in the measurements

of the A-C circulation by the cyanide method is not the case. Since the velocity of the circulation is reported as slowed in the capillaries in normal pregnancy (38, 57) there need be no contradiction in the results. Klee also reported a difference between primiparae and multiparae but since only single observations during the last three months of pregnancy were made his results are difficult to evaluate. Such a difference was not demonstrated in this series.

In a study by Spitzer of the circulation time in pregnancy, the use of the decholin method indicated no difference in the arm to tongue pathway between normal non-pregnant and pregnant women. In that study single observations were made on 27 normal patients, all in the last three months of pregnancy. This fact, plus the possible effect of the altered sensibility of the tongue in pregnancy (49) make the results from that study difficult to compare with those obtained by the cyanide method.

The values for pulmonary circulation time fall, without exception, within the normal non-pregnant limits of 7 to 14 seconds. Its curve follows quite closely that of the average A-C time (Figures 4, 7, 8), although the changes are slight. Between it and the pulse rate and hemoglobin (individual observations) no evident correlation exists (Figures 5 and 6). X-ray photographs of the lungs during normal pregnancy show an increase in the pulmonary markings (58) which might be interpreted as being due to increase in the size of the capillary bed. If this is true, it would account for the relatively slight increase in the velocity of the pulmonary blood flow as contrasted with that observed in the A-C pathway. It would also account for the increase in total amount of blood flowing through the lungs, coincident with the increase in cardiac output, without an increase in the velocity of pulmonary flow.

Since the pulmonary circulation time shows but slight change throughout pregnancy and the puerperium, the venous circulation time follows the changes observed in the A-C time and the factors influencing it are the same as those discussed in relation to the A-C circulation time. The speeding of the circulation apparently occurs in the component of the circulatory pathway and not in the pulmonary circuit.

The *vital capacity, blood pressure, pulse rate, respiratory rate, venous pressure, and subcostal angle* showed no apparent relationship to the changes observed in the circulation time and need not be discussed here.

Neither dyspnea or edema bore any constant relationship to the changes in the velocity of blood flow (Table I).

#### CONCLUSIONS

1. The arm to carotid, pulmonary and venous circulation times can be studied safely in pregnancy by the cyanide method.

2. The values for the arm to carotid, pulmonary and venous circulation times fall within the normal non-pregnant range.

3. There is a decrease in the average arm to carotid circulation time from the 17th to the 36th week of pregnancy, inclusive.

4. There is probably an increase in the average arm to carotid circulation time, relative to the 17th to 36th week period, in the period from the 37th to the 40th week.

5. There is a decrease in the average arm to carotid circulation time following delivery which persists until the 7th postpartum week; after which the arm to carotid circulation time returns to the normal non-pregnant level.

6. There is little change in the pulmonary circulation time during pregnancy, although the trend is the same as that of the arm to carotid circulation time.

7. The speeding of the circulation in pregnancy seems to occur in the peripheral venous component of the vascular system.

8. Various factors that might decrease the circulation time in pregnancy are discussed, and, of them, the decreased viscosity of the blood is considered the most probable important contributing factor.

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# THE BLOOD LIPIDS OF DIABETIC CHILDREN<sup>1</sup>

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The development of degenerative changes in the cardiovascular system in diabetes has focused attention in recent years on the lipid metabolism of diabetes in childhood, a period in which diabetes occurs in a less complicated form than in the adult. Despite the fact that satisfactory evidence is still lacking for proof, there is a belief that the arteriosclerosis observed today even in the young diabetic is related to the cholesterol level of the blood. The growing importance of the diabetic child, particularly since today he provides a source from which the adult diabetic is recruited, makes highly desirable the accumulation of data on various aspects of his lipid metabolism. In the present investigation, therefore, a study has been made of all blood lipid constituents, namely, total fatty acids, phospholipids, and free and esterified cholesterol, in diabetic children under controlled conditions. Although lipids in the blood of diabetic children have previously been reported (1, 2, 3), these studies are few in number. Some of them have employed unsatisfactory methods or have dealt with a single lipid constituent, i.e., total cholesterol, which a number of workers regard as an index of the level of other lipid constituents in the blood.

## EXPERIMENTAL

Forty-nine children were employed in this study. Twenty-three of them were non-diabetic and were used to establish the normal lipid level. The latter were school children who came to the Outpatient Department for routine tests, and in whom no abnormalities—unless otherwise recorded—were found by clinical examinations. No attempt was made to regulate the diet or nutritional state of these normal subjects other than

withholding all food for approximately 12 to 14 hours prior to removal of the blood sample.

In 3 cases blood was obtained from the diabetic children at a time when acidosis was present. The remainder of the observations, 26 in all, were made on children under adequate insulin and dietary control. At the time blood was taken for lipid analyses these patients had either been hospitalized for some time or been admitted for a single day for routine laboratory and physical examination as well as for regulation of diet and insulin dosage, a procedure that was carried out at intervals of 2 or 3 months.

Whole blood was used for lipid analyses, and the oxidative methods employed have been previously reported (4). The determinations of blood lipids were carried out in triplicate; the values recorded are the averages of closely agreeing figures.

Okey and Stewart (5) pointed out several years ago that irregularities in the effects produced by anticoagulants and centrifugation make plasma less desirable than whole blood for comparative lipid studies. The errors introduced in the lipid determinations of plasma obtained by the use of oxalate have been studied more recently by Schmidt (6) and by Sperry and Schoenheimer (7), who have shown that oxalated blood plasma contains smaller amounts of phospholipids and cholesterol than heparinized plasma. Despite the unequal distribution of cholesterol between plasma and corpuscles, there seems little justification at the present time for the claim that plasma or serum provides a more significant medium for lipid determination than whole blood. Too little is known of the rôle of the corpuscles in lipid metabolism, particularly in pathological conditions. Hence, in the present investigation, whole blood was used throughout for the comparison of the lipid content of the blood of normal and diabetic children.

<sup>1</sup> Aided by a grant from the Pauline Fore Moffitt Fund for the study of Juvenile Diabetes.

TABLE I  
Whole blood lipids (postabsorptive) of controlled diabetic children

Case number	Date blood taken		Age	Weight	Insulin	Diet			Cholesterol				Phos- pho- lipids	Total fatty acids	Resid- ual fatty acids*	Total lipid
						Fat	Car- bohy- drate	Pro- tein	Total	Free	Ester					
	1934		years	kgm.	units	grams	grams	grams	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent of total	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
A1	August	18	14	42.5	5-0-15	150	75	90	181	101	80	44	256	370	140	551
A2	August	18	4	18.6	4-0-4	115	50	60	157	106	51	32	231	250	58	407
A3	August	22	8	22.0	10-0-10	120	50	60	165	111	54	33	264	315	99	480
A4	September	6	10	34.5	20-0-0	100	200	70	194	113	81	42	244	331	108	525
A5	September	15	15	54.5	10-5-10	200	80	90	170	119	51	30	231	301	109	471
A6	September	22	16	43.0	17-0-13	80	90	65	187	114	73	39	229	304	97	491
A7	September	22	13	51.2	15-10-15-10	92	150	60	205	133	72	35	246	356	138	561
A8	October	13	13	34.6	10-8-8-5	160	70	70	181	115	66	36	237	305	98	486
A9	October	27	4.5	18.9	6-0-7	95	35	65	189	125	64	34	213	264	74	453
A10	October	27	6	19.8	6-4-5-3	65	100	50	182	118	64	35	237	312	106	494
A11	October	27	8	24.5	10-0-10	100	60	60	178	112	66	37	348	385		526
A12	November	17	9	26.8	7-3-9-3	145	65	70	221	136	85	38	385	385		606
A13	November	17	14	47.6	18-14-16-11	144	160	65	171	96	75	44	248	356	135	527
A14	December	8	14	44.0	15-10-15-12	100	100	50	203	129	74	36	247	317	97	520
A15	January	9	10	31.0	14-10-12-4	110	150	60	152	113	39	26	227	314	133	466
A16	March	2	13	34.1	20-10-30	140	90	70	177	111	66	37	391	356		568
A17	March	2	15	59.4	15-10-15	195	100	90	184	122	62	34	287	356	119	540
A18	March	23	12	34.1	8-0-8	205	90	70	162	106	56	35	270	354	132	516
A19	April	20	19	57.3	25-0-25-15	165	100	75	198	101	97	49	280	395	136	593
A20	March	30	5	20.0	0-0-0	100	50	50	162	106	56	35	263	353	136	515
A21	May	11	10	28.7	10-0-8	160	70	55	174	107	67	38	240	330	120	504
A22	July	27	13	35.0	16-10-16-10	150	80	64	173	107	66	38	281	337	101	518
A23	August	28	12	31.0	5-0-5	145	70	60	211	116	95	45	301	409	138	620
A24	August	28	7	26.7	8-0-6	127	55	60	146	97	49	34	249	292	89	438
A25	September	7	5	17.7	10-0-8	110	50	55	192	105	87	45	237	318	95	510
A26	January	12	9	35.2	8-5-8	130	70	50	168	111	57	34	242	313	109	481
Maximum									221	136	97	49	301	409	140	620
Minimum									146	96	39	26	213	250	58	407
Mean									181	113	68	37	250	334	112	514

\* Derived chiefly from neutral fat.

## RESULTS

The various lipid constituents determined in the whole blood of normal children are shown in Table II, whereas the values for the diabetic children are recorded in Tables I and III. The results obtained for the latter may be grouped according to the degree of control effected by insulin and diet.

1. *Controlled diabetic children.* The concentration of all lipid constituents that was found in the blood of the controlled diabetics corresponded closely to the normal range. Thus the maximum and minimum values for total lipids were respectively 620 and 407 mgm. per cent as compared with values of 595 and 417 mgm. observed in normal subjects. The total fatty acid content of the blood of diabetic children varied from 409 to 250 mgm. per cent, whereas the highest and lowest values for this constituent in the normal children were respectively 387 and 260 mgm. per cent. The phospholipid values fluctuated between 301 and 213 mgm. in the diabetic and between 288 and 184 in the normal children. The close agreement

between the cholesterol values of normal and diabetic subjects is particularly striking. Total cholesterol, which was present to the extent of 226 to 141 mgm. per 100 cc. of the blood of the normal children, ranged from 221 to 146 mgm. per cent in the diabetics; the free or uncombined portion of this consisted of 131 to 91 in the non-diabetics and 136 to 96 in the diabetics. The mean values obtained for both groups of children are also in close agreement.

2. *Diabetic children in acidosis.* Although this study was concerned primarily with children under control, blood lipids were also obtained from 3 cases suffering from diabetic acidosis (Table III). In 2 of these (A19 and A21) the postabsorptive blood samples obtained during acidosis contained a much higher concentration of total lipids than samples taken during periods in which these patients were under control. The various lipid constituents, however, did not share equally in this rise of the total lipid. While no increase in cholesterol was found in A21 during acidosis, in A19 it rose from a controlled level of

TABLE II  
Whole blood lipids (postabsorptive) of non-diabetic children

Case number	Date blood taken	Age	Weight	Cholesterol				Phospho- lipids	Total fatty acids	Residual fatty acids	Total lipid
				Total	Free	Ester					
	1886	years	kgm.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent of total	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
C3	Feb. 9	13		146	96	50	34	240	277	80	423
C4	Feb. 9	6	21.6	198	131	67	34	254	325	106	523
C5	Feb. 9	5	17.7	162	107	55	34	253	297	88	459
C6	Feb. 16	14	45.1	173	105	68	39	217	318	123	491
C7	April 13	13	47.2	164	105	59	36	268	304	81	468
C8	April 20	15	49.5	141	91	50	35	222	279	93	420
C9	July 7	4	17.2	154	105	49	32	259	320	110	474
C10	July 7	8	25.2	152	100	52	34	273	325	104	477
C11	Aug. 3	13	38.4	141	94	47	33	249	276	75	417
C12	Sept. 7	15	47.2	158	95	63	40	230	323	123	481
C13	Sept. 7	7	20.9	183	107	76	42	278	298	57	481
C14	Sept. 21	5	15.8	176	96	80	46	219	334	129	510
C15	Sept. 21	9	27.5	181	104	77	43	240	260	43	441
C16	Oct. 26	10	30.0	174	104	70	40	248	280	63	454
C17	Nov. 18	12	31.8	161	108	53	33	220	279	93	440
C18	Nov. 25	12	34.6	150	98	52	35	184	313	152	463
C19	Nov. 25	13	35.6	172	94	78	43	186	288	107	460
C20	Nov. 25	11	35.2	150	100	50	33	186	290	129	440
C21	Dec. 16	12	36.0	208	126	82	39	270	387	147	595
C22	Dec. 16	9	29.3	164	96	68	41	288	338	95	502
C24	Dec. 23	4	14.7	169	92	77	46	218	261	58	430
C25	Dec. 23	7	23.6	198	109	89	45	214	336	127	534
C26	Dec. 23	6	19.7	226	122	104	46	212	283	66	509
Maximum				226	131	104	46	288	387	152	595
Minimum				141	91	47	32	184	260	43	417
Mean				170	104	66	38	236	304	98	474

198 to 238 mgm. per cent during acidosis. But it should be noted that the latter value does not represent a significant rise above the highest normal, namely 226, or for that matter above the highest value found in the controlled diabetics, namely 221 mgm. per cent. Changes in the cho-

lesterol ester or phospholipid content of the blood during acidosis were neither marked nor consistent in these 2 cases.

A6 was brought into control by means of a daily injection of 42 units of insulin and a diet containing 90 grams of fat, 84 grams of carbo-

TABLE III  
Blood lipids of diabetic children in acidosis. (Blood samples taken in the postabsorptive state unless otherwise stated)

Case number	Date	Condition	Cholesterol				Phospho-lipid	Total fatty acids	Residual fatty acids	Total lipid	Insulin	Diet		
			Total	Free	Ester							Fat	Carbohydrates	Protein
			mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent of total	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	units	grams	grams	grams
A6	July 13, 1935	Controlled	163	101	62	38	258	319	101	482	42	90	84	74
	July 20, 1935	Acidosis	182	112	70	38	281	359	120	541	18	90	84	74
	July 25, 1935	Acidosis*	196	113	83	42	389	967	648	1165	26	90	84	74
	July 26, 1935	Acidosis	174	116	58	33	289	356	120	530	95	90	84	74
	July 27, 1935	No acidosis	193	116	77	40	291	375	124	568	40	90	84	74
	August 3, 1935	No acidosis	184	113	71	39	281			56	90	84	74	74
	August 28, 1936	Controlled	170	109	61	36	278	356	125	526	82	90	84	74
	September 7, 1936	Controlled	160	98	62	39	264	313	92	473	42	40	120	74
	October 5, 1936	Controlled	159	97	62	39	261	322	102	481	24	67	60	74
A19	March 30, 1935	Acidosis	238	129	109	46	268	730	470	968		75	100	75
	April 20, 1935	Controlled	198	101	97	49	280	395	136	593	65	75	100	75
A21	April 29, 1935	Acidosis	165	104	61	37	296	576	333	742		160	70	55
	May 11, 1935	Controlled	174	107	67	38	240	330	120	504	20	160	70	55

\* Blood sample obtained at 3:00 p.m.

hydrate and 74 grams of protein. From July 14 to 27 the insulin was gradually reduced so that by July 18 he was receiving 18 units, and this was continued until July 25. Blood lipids were examined on 3 different occasions during the period in which the patient showed acetonuria. Slight rises in total cholesterol occurred, but these were in no way marked when compared with the values obtained several days later when the patient was free of acetonuria. Indeed, the highest value observed during acidosis was 30 mgm. per cent below the highest figure found among the normal children. In 2 of the 3 blood samples taken during acidosis (July 20 and 26) small increases were noted in phospholipids and neutral fat. But again, if these values are compared with those obtained a few days later when the acidosis had disappeared, or with the highest values obtained in the normal subjects, it is questionable whether much significance can be attached to such increases. A striking rise in these 2 lipid constituents did occur, however, in the blood examined at the height of acidosis (July 25), but since this sample was obtained at 3 p.m. instead of in the postabsorptive state, it is difficult to differentiate between the effects of the 2 previous meals and those of the acidosis. It should be noted, however, that, as judged by previous observations made in this laboratory, such increases in neutral fat are not encountered in a normal alimentary lipemia (8).

#### DISCUSSION AND SUMMARY

The frequency with which the diabetes of the adult and elderly subject is associated with other pathological conditions makes difficult the interpretation of studies made in these age periods. This difficulty, however, is not so frequently met in the diabetes of childhood. In the group of diabetic children reported in this investigation, abnormalities other than diabetes were not present at the time lipid studies were made. The diabetic child thus provides the most satisfactory patient from whom metabolic disturbances due to diabetes per se may be deduced. The results of the present study show quite definitely that, when controlled by insulin and diet, diabetic children have blood lipid levels well within the normal range. This was found to be the case with all

lipid constituents, i.e., free and esterified cholesterol, phospholipids and fatty acids. Normal lipid values were found whether the diabetes was of 3 months' or 3 years' duration, and whether the subject required 68 or 10 units of insulin. These observations on children are thus in accord with the results recently reported by Man and Peters (9) for adult and elderly patients. They found no relation between serum cholesterol and the severity of the diabetes as judged by insulin requirement or carbohydrate tolerance.

From a comparison of the values obtained from 3 patients during acidosis and control, it is apparent that a pronounced increase in the cholesterol content of the blood need not accompany mild acidosis in diabetic children. In no case during acidosis was a value found significantly above the highest normal. Despite the fact that a high cholesterol level has been suggested as a precursor of complications, nevertheless a number of observations now indicate that the cholesterol content of the blood is not a reliable index of the degree of control in diabetes. Thus in 65 of their 94 cases of acidosis, White and Joslin (10) found cholesterol values within or slightly above the normal range. Moreover, Man and Peters (11) have shown that cholesterol falls below the acidosis level during the period immediately following the disappearance of acidosis and dehydration, and that at this time the cholesterol content of the blood may be even lower than at the end of convalescence.

While neutral fat, the changes in which are reflected in the total fatty acid determination, fluctuated widely at times, commensurate changes did not always occur in the cholesterol fraction. The most striking example of this was found in A6 and A21 (Table III). Over a period of 5 days, residual fatty acids rose from 120 to 648 mgm. per cent (A6) at the same time that total cholesterol rose from 182 to 196 mgm. Although 2 days later residual fatty acids dropped to 124, cholesterol still remained practically unchanged. A similar lack of relation between cholesterol and total fatty acids has been observed by others (11, 12). It follows, therefore, that cholesterol cannot be employed as a guide for the level of other lipid constituents in the blood.

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# HEMATOLOGICAL STUDIES IN HYPOTHYROIDISM FOLLOWING TOTAL THYROIDECTOMY<sup>1</sup>

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The frequent occurrence of mild anemia in myxedema has long been recognized (1, 2). The red blood cell count has usually been between 3,000,000 and 4,500,000 per cubic millimeter with hemoglobin percentage occasionally as low as 60 per cent but most often approximating 75 per cent. There is less agreement concerning changes in white blood cell count, different authors describing leukocytosis, leukopenia, or a normal white count. It was felt that more precise data concerning the nature and extent of the blood changes could be obtained from the study of patients in whom hypothyroidism was developing under close observation. Accordingly, studies were made before and after total thyroidectomy in patients with chronic heart disease (3, 4, 5). The blood counts, hemoglobin percentages, arterial oxygen capacities, and type and size of red and white blood cells in relation to changes in the basal metabolic rate during the development and control of hypothyroidism induced by total thyroidectomy are reported below. The effects of iron and of thyroid medication on the blood findings in postoperative hypothyroidism are also described.

## METHODS

Red and white blood cell counts and hemoglobin estimations were made from capillary blood in a series of 40 patients at various levels of metabolism after total thyroidectomy. In nine additional patients more extensive hematological studies on venous blood were made before and at varying time intervals after total thyroidectomy; six of these patients were operated on for the relief of congestive heart failure and three for angina pectoris. In a further group of fifteen patients with previous congestive failure or angina pectoris, seventeen studies were done postoperatively on the venous blood. The preoperative studies were made a few days before operation when patients with congestive failure

showed minimal signs of decompensation and were in the best possible condition for operation; all postoperative studies were made a few weeks to three years following thyroidectomy in the absence of signs of congestive heart failure. In many instances, patients studied postoperatively were receiving doses of desiccated thyroid sufficient to prevent distressing symptoms of myxedema, basal metabolic levels of approximately —20 to —30 per cent being maintained (6).

Except in the preliminary studies, oxalated venous blood drawn without stasis from the patients in the postabsorptive state, was used for measurement of hemoglobin per cent and red and white blood cell counts. Icteric indices and hematocrit readings were estimated from the same samples of blood, a correction factor of 1.08 being applied to the hematocrit reading for the amount of oxalate used (7). Smears for Schilling index and measurements of mean cell diameter and for reticulocyte and platelet counts were made from capillary blood. Measurements of oxygen capacity were made on arterial blood drawn from the patients in the postabsorptive state.

Hemoglobin measurements were made by the Sahli method using a Sahli hemoglobinometer standardized so that 100 per cent hemoglobin corresponded to 20.9 volumes per cent oxygen capacity. The oxygen capacity was measured by the method of Van Slyke and Neill (8). Red and white blood cell counts were done in duplicate using the same counting chamber and cover slip throughout. Pipettes and counting chamber had been calibrated by the United States Bureau of Standards. The reticulocytes and platelets were estimated by the method of Dameshek (9). A calibrated micrometer eye-piece was used for measuring mean cell diameters; mean corpuscular volumes were calculated from the hematocrit per cent and the red blood cell count. Icteric indices were estimated by the method of Davis (10). Measurements of basal metabolic rate were made in duplicate with a Collins-Benedict-Roth apparatus and calculated according to the Aub-DuBois normal standards (11).

## RESULTS

The preliminary studies on capillary blood showed a decrease in the hemoglobin percentage and the red blood cell count at low levels of basal metabolism following total thyroidectomy (Table I). This finding was more striking in patients operated upon for the relief of congestive heart

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TABLE I

*Average results of postoperative blood counts on capillary blood*

Deviation from normal basal metabolic rate	Number of counts	Hemoglobin (Sahli)	Red blood cells	Number of counts	White blood cells
<i>per cent</i>		<i>per cent</i>	<i>per cu.mm.</i>		<i>per cu.mm.</i>
PATIENTS WITH CONGESTIVE HEART FAILURE					
+ 2 to -10	6	93	4,367,000	3	9,250
-10 to -20	9	90	4,387,000	2	10,400
-20 to -30	11	87	3,948,000	4	7,662
-30 to -40	11	80	3,874,000	7	7,050
PATIENTS WITH ANGINA PECTORIS					
0 to -10	5	89	4,114,000	2	10,800
-10 to -20	9	95	4,685,000	4	8,025
-20 to -30	13	94	4,351,000	3	6,066
-30 to -40	12	89	4,168,000	5	7,425

failure than in those operated upon for the relief of angina pectoris. The changes in hemoglobin and red blood cell count were paralleled, in general, by changes in oxygen capacity; twenty-three de-

TABLE II

*Correlation between arterial oxygen capacity and basal metabolic rate following total thyroidectomy*

Case	Deviation from normal basal metabolic rate	Oxygen capacity
	<i>per cent</i>	<i>volumes per cent</i>
W. M.	+1	20.8
	-31	16.5
A. R.	-2	20.8
	-34	17.7
E. P.	-23	17.1
	-20	18.1
M. W.	-7	21.9
	-19	18.3
M. C.	-24	18.4
	-34	15.7
E. M.	-4	22.0
	-34	20.7
G. M.	-6	23.9
	-11	22.5
	-24	21.7
G. B.	-9	19.8
	-15	17.9
M. H.	+1	22.6
	-7	22.5
	-21	20.1
T. K.	-1	21.6
	-11	19.7
	-28	18.9

terminations of arterial oxygen capacity done on ten patients before and after total thyroidectomy showed a diminution of oxygen capacity as the basal metabolic rate decreased (Table II).

Hematological studies made before and at various time intervals after operation in six patients operated on for the relief of congestive failure revealed a lowering of the hemoglobin per cent, the hematocrit reading, the red and white cell and platelet counts and an increase in the mean corpuscular volume and mean cell diameter when the basal metabolic rate had fallen to low levels (Table III). In Case T. K. (Table III), who showed a typical drop in basal metabolic rate from -1 per cent preoperatively to -29 per cent three months after operation, the red blood cell count decreased from 4,450,000 to 3,760,000, the hemoglobin per cent from 90 to 87, the white blood cell count from 5,700 to 3,050, the hematocrit per cent from 45.3 to 38.9, the platelet count from 293,000 to 289,000; the mean corpuscular volume showed a rise from 101 to 103.4, and the mean cell diameter increased from 7.7 to 8.0. Six months after operation, although the basal metabolic rate was essentially the same (-28 per cent), the blood showed further change. These data are graphically presented in Figure 1. Data obtained in eleven postoperative studies on ten other patients operated upon for the relief of congestive failure corroborate the above findings (Table III). In Table IV, the data obtained postoperatively in all the patients operated upon for congestive failure are divided into four groups representing various levels of basal metabolism, and the average values for each group are recorded. Despite considerable individual variation, a lowering of the average values for hemoglobin, red and white blood counts, hematocrit per cent, and platelet count, and an increase in the values for mean corpuscular volume and mean cell diameter were evident as the basal metabolic rate decreased (Figure 2). In general, the corpuscular volume increased more than the cell diameter, so that the red blood cells apparently became thicker.

In one patient (Case E. B., Table III) a hypochromic anemia due to frequent hemoptyses was present before thyroidectomy. Two months after operation the hemoglobin was 101 per cent and the

TABLE III  
Studies of hematology following total thyroidectomy

Case	Diagnosis	Time of study ‡	Devi- ation from normal metabol- ic rate	Hemo- globin (Sahli)	Red blood cells	White blood cells	Hema- to- crit	Icteric index	Re- ticu- lar cytes	Platelets	Mean cor- puscular volume	Mean cell di- ameter	Differential count				
													Polys	Lymphs	Eosino- phils	Baso- phils	Monocytes
B. C.	C. H. F.*	Pr. 3 weeks po.	+11 -4	102 102	5,800,000 5,105,000	7,475 9,875	50.6 47.0	6 4	0.4 0.3	610,000 556,445	87 92	7.4 7.5	86.5 86.0	11.5 12.5	0.5 0.0	0.0 0.0	1.5 1.5
G. B.	C. H. F.	Pr. 14 weeks po.	-9 -15	96 78	4,960,000 4,050,000	6,130 7,925	48.0 38.4	4 4	0.2 0.2	238,000 267,000	97 95	7.7 7.2	61.0 70.0	34.5 20.0	1.5 2.5	0.0 0.5	3.0 7.0
M. H.	C. H. F.	Pr. 2 weeks po. 3 weeks po.	+1 -15	89 90	4,325,000 4,750,000 4,195,000	6,575 7,250 6,650	49.1 47.0	10 6	1.5 0.1 0.0	363,000 294,000 264,000	113 99	7.7 7.4	71.5 75.0 70.0	25.0 22.0 23.5	2.0 0.0 1.5	0.5 0.0 1.0	1.0 4.0 4.0
T. K.	C. H. F.	Pr. 3 months po. 6 months po.	-1 -28 -26	90 87 73	4,450,000 3,760,000 3,380,000	5,700 3,050 2,500	45.3 38.9 38.3	10 8 12	0.9 0.0 0.1	293,000 289,200 203,000	101 103 113	7.7 8.0 7.9	76.0 51.5 58.0	19.0 41.5 32.5	2.5 3.5 3.5	1.0 0.5 1.5	1.5 3.0 3.5
J. K.	A. P.†	Pr. 3 months po.	-4 -17	76 83	4,156,000 3,945,000	6,250 6,150	39.0 38.9	8 9	0.0 1.0	220,000 371,000	94 99	7.3 7.7	55.5 52.5	40.5 46.0	0.0 0.0	1.0 0.0	3.0 1.5
E. R.	A. P.	Pr. 6 months po.	+2 -19	97 93	4,905,000 4,640,000	7,075 7,175	44.3 44.8	6 6			90 97	7.4 8.2	59.5 83.5	35.0 14.0	2.0 1.0	1.0 0.0	2.5 1.5
J. P.	A. P.	Pr. 4 months po. 7 months po.	-7 -27 -24	92 89 85	4,405,000 3,720,000 3,655,000	5,825 5,575 4,100	40.5 37.3 43.7	6 4 8	0.1 0.1 0.0	167,500 268,000 186,400	92 100 120	7.7 7.8 7.6	65.0 60.5 65.5	32.5 35.5 30.0	0.0 1.5 1.5	0.5 0.0 0.0	2.0 2.5 3.0
A. C.	C. H. F.	Pr. 4 months po. 6 months po.	+8 75	84 85	4,020,000 3,450,000 3,460,000	7,625 5,925 5,325	38.9 36.2 38.3	8 10 8	0.2 0.1 0.2	245,000 189,000 221,000	97 105 111	7.8 7.9 7.7	61.0 75.0 75.0	32.5 21.5 19.5	1.5 0.5 2.5	0.0 0.5 1.5	5.0 2.5 1.5
E. B.	C. H. F.	Pr. 1 month po. 2 months po. 3 months po.	-4 -18 -11 -19	72 82 101 70	4,200,000 3,970,000 5,100,000 3,655,000	7,025 6,075 8,950 4,150	39.4 38.9 47.5 35.7	2 4 8 6	0.2 0.4 0.1 0.1	428,000 497,000 300,900 168,000	94 78 93 98	8.0 7.6 7.6 7.5	65.5 66.5 61.5	28.5 28.5 30.5	3.0 0.5 1.5 4.0	0.5 0.5 2.0 0.5	2.5 2.0 4.0 3.5
M. P.	A. P.	10 months po.	-32	88	3,950,000	6,825	43.5	4	0.0	252,800	110	7.2	61.0	37.5	1.0	0.0	0.5
F. D.	C. H. F.	15 months po.	-34	78	3,835,000	6,800	36.2	2	0.0	295,000	94	7.9	59.0	37.0	2.5	0.0	1.5
S. F.	A. P.	11 months po. 12 months po.	-42	102	4,315,000 4,210,000	6,425 7,425	45.3 43.0	2 6	0.0 0.2	353,000 273,000	105 104	7.8 7.8	77.5 61.0	29.5 33.5	0.5 2.0	0.5 0.5	1.5 3.0
W. D.	C. H. F.	19 months po.	-30	80	3,865,000	6,000	37.3	2	0.0	251,000	97	7.0	69.5	27.0	1.0	0.5	2.0
M. G.	C. H. F.	12 months po.	-13	78	3,820,000	5,850	36.7	6	0.2	359,000	96	7.6	50.0	41.5	1.5	1.5	5.5
W. Da	C. H. F.	8 months po.	-20	71	3,340,000	5,875	32.9		0.0	337,000	99	7.8	71.5	18.5	2.5	0.5	7.0
B. Z.	C. H. F.	20 months po.	-34	83	3,080,000	3,850	32.9	4	0.2	333,000	107	7.6	80.0	15.5	1.0	0.5	3.0
M. S.	A. P.	7 months po.	-19	95	5,305,000	11,025	43.2		0.0	430,000	81	7.6	62.5	33.5	1.0	0.5	2.5
M. W.	A. P.	16 months po.	-29	82	3,785,000	6,575	40.5	6	0.0	223,000	107	7.5	51.5	45.0	0.0	0.0	3.5
E. M.	C. H. F.	12 months po.	-17	93	4,510,000	9,725	45.4	6	0.4	270,000	101	7.2	82.0	14.5	1.0	0.5	2.0
L. M.	C. H. F.	18 months po.	-19	96	4,865,000	5,225	45.9	6			94	7.6	69.0	28.0	1.0	0.0	2.0
M. F.	A. P.	18 months po.	-27	97	4,530,000	4,775	44.8	4	0.3	326,000	99	7.5	49.5	47.5	1.5	0.5	1.0
G. F.	C. H. F.	25 months po. 26 months po.	-27 -29	74 75	3,435,000 3,405,000	5,025 4,850	35.1 35.2	6 6	0.1 0.4	209,600 187,275	102 103	7.3 7.6	54.5 58.0	41.0 41.0	0.0 0.0	0.0 0.0	4.5 1.0
H. W.	C. H. F.	12 months po.	-23	78	3,900,000	8,050	36.5	4	0.1	242,000	94	7.7	67.5	28.5	0.5	0.0	3.5
H. G.	C. H. F.	18 months po.	-27	79	3,570,000	6,250	35.0	6	1.0	179,500	99	7.4	51.0	45.5	0.5	0.5	2.5

\* C. H. F. refers to congestive heart failure. † A. P. refers to angina pectoris. ‡ Pr. = preoperative. Po. = postoperative.

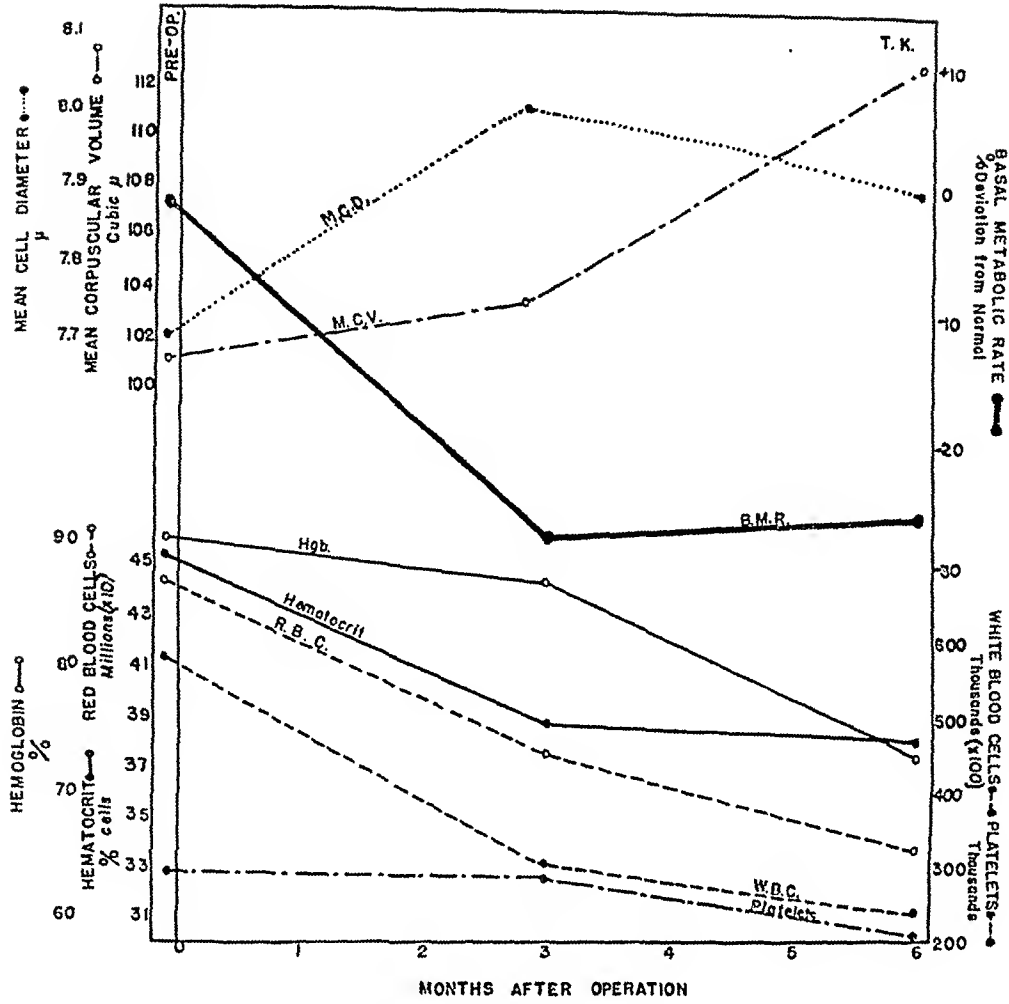


FIG. 1. RELATION BETWEEN TIME OF ONSET OF HYPOTHYROIDISM AND OF CHANGES IN BLOOD PICTURE FOLLOWING TOTAL THYROIDECTOMY (CASE T. K.).

TABLE IV  
Average results of postoperative studies in hematology. Studies on venous blood

Deviation from normal basal metabolic rate*	Number of studies	Hemo-globin (Sahli)	Red blood cells	White blood cells	Hematocrit	Platelets	Mean corpuscular volume	Mean cell diameter
per cent		per cent	per cu.mm.	per cu.mm.	per cent	per cu.mm.	cu. μ	μ
PATIENTS WITH CONGESTIVE FAILURE								
*Preop. level	6	89	4,630,000	6,755	45.2	366,000	96	7.7
0 to -10	2	100	4,927,000	8,563	47.0	425,000	96	7.4
-10 to -20	6	86	4,402,000	6,908	42.3	331,000†	94	7.4
-20 to -30	7	77	3,534,000	5,086	36.0	235,000	102	7.7
-30 to -40	3	80	3,593,000	5,550	35.5	293,000	99	7.5
PATIENTS WITH ANGINA PECTORIS								
Preop. level	3	88	4,489,000	6,380	41.3	194,000‡	92	7.5
-10 to -20	3	90	4,630,000	8,117	42.3	400,000‡	92	7.8
-20 to -30	4	88	3,947,000	5,256	41.6	251,000	107	7.6
-30 to -40	1	88	3,950,000	6,825	44.0	253,000	110	7.2

\* The range of preoperative basal metabolic rate in all patients was from +11 to -9 per cent.  
† Represents average of 5 observations.  
‡ Represents average of 2 observations.

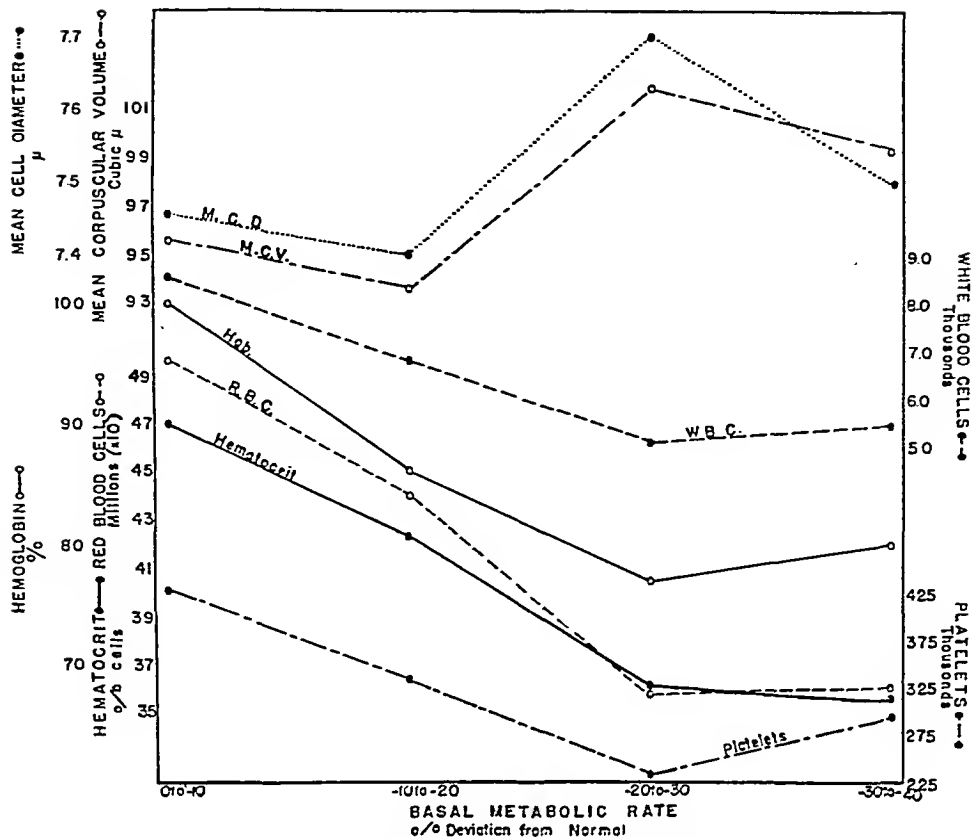


FIG. 2. RELATIONSHIP BETWEEN CHANGES IN HEMATOLOGY AND CHANGES IN BASAL METABOLIC RATE FOLLOWING TOTAL THYROIDECTOMY.

Summary of average results in eighteen patients operated on for congestive heart failure.

red blood cell count, 5,100,000. Subsequently, as hypothyroidism persisted, a normocytic anemia developed.

Studies before and at various time intervals after thyroidectomy in three patients operated on for the relief of angina pectoris revealed no significant hematological changes in two of these (Table III). The third patient developed a slight anemia when the basal metabolic rate had dropped from  $-7$  per cent preoperatively to  $-24$  per cent postoperatively. Four additional studies done postoperatively only on four other patients operated on for angina pectoris confirmed these findings; only two of these patients showed evidence of slight anemia at basal metabolic rates of  $-29$  per cent and  $-32$  per cent (Table III). The average results of the studies in patients operated on for angina pectoris are recorded in Table IV.

Although the white blood cell count in many instances was significantly decreased when hypothyroidism had developed, no striking deviations from the normal distribution of the various cell types were observed in differential and Schilling index counts in any of the patients studied (Table III). The red blood cells appeared normal in size and shape; there was no achromia. The reticulocyte counts varied within normal limits. Icteric indices ranged from 2 to 12; values above 8 were encountered in only three patients operated on for congestive heart failure and in only one patient operated on for angina pectoris; all values were within the normal limits.

Studies of the hemoglobin per cent, red blood cell count, and white blood cell count were made on four patients with postoperative hypothyroidism before the institution of iron therapy and again after patients had been receiving this medi-

TABLE V  
*Effect of medication on anemia of hypothyroidism*

Case	Deviation from normal basal metabolic rate	Hemoglobin (Sahli)	Red blood cells	White blood cells	Medication
	<i>per cent</i>	<i>per cent</i>	<i>per cu.mm.</i>	<i>per cu.mm.</i>	
G. F.	-27	74	3,435,000	5,025	Before medication.
	-29	72	3,730,000		Ferrous sulfate grains 4, 6 i. d. for 3 weeks.*
	-29	75	3,405,000	4,850	Ferrous sulfate grains 4, 6 i. d. for 4 weeks.
L. B.	-29	90	3,950,000	5,000	Before medication.
	-27	94	4,150,000		Ferrous sulfate grains 4, 6 i. d. for 2 months.
S. L.	-27	85	3,980,000		Before medication.
	-32	78	3,520,000		Reduced iron 15 grains t. i. d. for several months.
H. W.	-23	78	3,900,000	8,050	Before medication.
	-17	85	4,140,000		4 grains iron 4 i. d.
G. F.	-29	75	3,405,000	4,850	Before medication.
	-27	75	3,430,000		Thyroid grains 1/20 daily for 1 month.†
W. D.	-31	80	3,865,000		Before medication.
	-23	91	4,480,000		Thyroid grains 1/10 daily for 2 months.

\* Daily reticulocyte count for 2 weeks following institution of iron therapy. No reticulocytosis.

† Daily reticulocyte count for 1 month following institution of thyroid medication. No reticulocytosis.

cation for one month or longer. No significant changes were found even when large doses of iron were given (Table V). Daily reticulocyte counts in one patient for three weeks following iron medication showed no increase in reticulocytes.

Hematological studies and measurements of the basal metabolic rates were made on two patients receiving small doses of desiccated thyroid (Armour's) for the alleviation of the more severe symptoms of myxedema. Case G. F. who received 1/20th grain thyroid daily showed no change in hematology and no significant change in basal metabolic rate over a period of four weeks after starting medication. Daily reticulocyte counts on this patient for one month following the institution of thyroid therapy showed no reticulocyte response. Another patient, Case W. D., received 1/10th grain thyroid daily for two months; there was an increase in hemoglobin from 80 per cent to 91 per cent and an increase in red blood cell count from 3,865,000 to 4,480,000 per cubic millimeter of blood when the basal metabolism had risen from -31 per cent to -23 per cent (Table V).

#### COMMENT

The data in the present study demonstrate the development of a mild anemia in hypothyroidism

following the total removal of the thyroid gland in man. When the basal metabolic rate had fallen to between -20 and -30 per cent, the average values found were 77 per cent hemoglobin and 3,534,000 red cells per cubic millimeter of blood in patients operated on for the relief of congestive heart failure, and 88 per cent hemoglobin and 3,948,000 red cells per cubic millimeter of blood in patients operated on for angina pectoris (Table IV). That the changes in the blood picture in patients with angina pectoris were not as striking as those in patients with congestive failure may be related to the fact that the majority of the subjects with angina had moderate degrees of pulmonary emphysema. The anemia in all patients after operation was at most moderate and usually only slight, the lowest values for hemoglobin and red blood count observed in the entire series being 71 per cent and 3,080,000 respectively (Table III). It should be noted that the patient with 71 per cent hemoglobin had a red blood cell count of 3,340,000 and the patient with 3,080,000 red cells per cubic millimeter of blood had a hemoglobin of 83 per cent. These observations are in harmony with reported findings in spontaneous myxedema in man and in hypothyroidism following experimental thyroidectomy in animals (1, 12, 13, 14, 15, 16).

Significant changes in the blood picture following total thyroidectomy occur only at low levels of basal metabolism. Data in one patient (Case T. K., Table III) indicate that the hemoglobin per cent and red blood cell count may continue to diminish slightly after the basal metabolic rate has become established at a low level. That more marked degrees of anemia were not encountered may be related to the fact that severe myxedema was not allowed to persist; small doses of desiccated thyroid were administered to alleviate the distressing symptoms of marked hypothyroidism, the basal metabolic rate being maintained at a level of between — 26 per cent and — 32 per cent in most instances.

None of the patients studied showed symptoms or physical signs which could be ascribed to the anemia. Sore tongue and paresthesias or objective neurological changes in the extremities were never encountered. The pallor and sensitivity to cold present in most cases in which the basal metabolic rate was below — 20 per cent were probably due to hypothyroidism as such. Elevation of the pulse rate was not observed in the cases of this series. Such dyspnea as was exhibited by the patients studied here was undoubtedly associated with their heart disease, and was invariably milder than before operation. Several observers have reported an increase in cardiac output (17, 18) or an accelerated velocity of blood flow (19) both indicative of increased cardiac work, in patients with low red blood cell counts and hemoglobin percentages. However, the decrease in oxygen carrying capacity of the blood in such instances was always more severe than that found in the patients studied here. Moreover, it has been shown that the cardiac output after total thyroidectomy is not influenced by the mild degrees of anemia which accompany a fall in basal metabolic rate to between — 20 per cent and — 30 per cent. It would appear, therefore, that the anemia which develops following total thyroidectomy is not clinically important and places no demonstrable burden on the cardiovascular system.

The progressive increase in color index, mean cell diameter, and mean corpuscular volume during the development of hypothyroidism indicate that the anemia tends toward the macrocytic hyperchromic type. The increase in thickness of

the red blood cells is somewhat greater than the increase in diameter. Kunde, Green, and Burns (16), and later Sharpe and Bisgard (20), in their studies on myxedematous rabbits, also observed a high color index and an increased diameter of the red blood cell. In one case of anemia of myxedema studied by Minot in 1921 (21), the size of the red blood cells was found to be normal and the color index slightly decreased. It is of interest that in one of the patients with mitral stenosis (Case E. B.), a hypochromic anemia, which was present before total thyroidectomy, cleared up entirely after operation when the repeated hemoptyses causing the anemia ceased. The red blood cell count and hemoglobin were 5,100,000 and 101 per cent respectively two months after operation. One month later, the blood showed evidences of a normocytic anemia.

The cause of the anemia of myxedema has not yet been established. Studies by other investigators of the effect of various medications have yielded data which support the concept of a decrease in hematopoiesis due to insufficient circulating thyroid hormone and not to a lack of Castle's anti-pernicious anemia factor or of iron. Baldridge and Greene (22) found no response to liver therapy in eleven patients with anemia of myxedema. Means, Lerman, and Castle (23) reported a case in which pernicious anemia, myxedema, and anemia of myxedema coexisted. The reticulocytosis after liver therapy was followed by a second reticulocyte response when thyroid medication was administered. Lerman and Means (24) felt that an iron deficiency was at least partly responsible for the anemia present in a few of their patients with spontaneous myxedema. It is, however, not entirely clear from their data that the iron deficiency was due to the hypothyroidism as such. In the present study iron deficiency was shown to be of no importance as an etiological factor in the anemia which developed after total thyroidectomy; four patients given moderate or large doses of iron showed no reticulocytosis and no significant changes in the blood picture.

Many observers have agreed that the anemia of myxedema is due to decreased formation of blood caused by lack of thyroid hormone. Minot wrote in 1921, "treat with active thyroid to clear up the myxedema and the anemia will improve con-

comitantly" (21). MacKenzie (14), and Stone (25), and Lerman and Means (24), have reported complete disappearance of the anemia of myxedema following appropriate thyroid medication. Lisser and Anderson (15) reported that under thyroid medication the improvement in the blood picture paralleled the restoration of the basal metabolic rate to normal. Kunde, Green and Burns (16) found a reticulocyte response of 4 to 10 per cent in hypothyroid animals following thyroid feeding.

The findings in the present study were in accord with those of other investigators. The response to minimal doses of thyroid was studied in two patients; in one instance, Case G. F. (Table V),  $\frac{1}{10}$ th grain desiccated thyroid was administered every second day with no significant change in basal metabolic rate or hematological findings. In another patient, Case W. D. (Table V), who was receiving  $\frac{1}{10}$ th grain thyroid daily a rise in basal metabolic rate from —31 per cent to —23 per cent occurred, and concomitantly there was an improvement in the anemia. These data support the concept that the anemia of myxedema is due to decreased bone marrow function associated with diminished circulating thyroid hormone.

Other evidences of diminished function of bone marrow were seen in the white blood cell and platelet counts. The white blood cell count was diminished when the basal metabolic rate decreased after total thyroidectomy; the average of seven values obtained in the patients operated on for congestive heart failure was 5,086 per cubic millimeter of blood when the basal metabolic rate was between —20 and —30 per cent. These data confirm the findings of Minot (21), Krantz (26), and Kocher (27) in spontaneous myxedema. However, other authors have found a normal (2, 25) or even an increased white blood cell count (12) in spontaneous myxedema. The findings of a decreased platelet count in many patients at low levels of metabolism after total thyroidectomy accords with the findings of Minot (21) in one patient with spontaneous myxedema. The lowered platelet count was in no case associated with purpuric manifestations.

Although diminished hematopoiesis occurs in hypothyroidism, the bone marrow retains its power to respond normally to stimuli. Unpublished results of studies made in this clinic show

that patients with the anemia of hypothyroidism following total thyroidectomy can respond to infection or anoxemia of congestive failure by a normal increase in white blood cell count and red blood cell count respectively.

### CONCLUSIONS

1. An anemia develops in hypothyroidism following total ablation of the normal thyroid gland for the alleviation of congestive heart failure and angina pectoris. The anemia tends toward the macrocytic hyperchromic type, being characterized by increased color index, mean corpuscular volume and mean cell diameter.
2. The anemia is at most moderate and usually only slight; it appears to be somewhat less marked in those patients operated on for angina pectoris than in those patients operated on for congestive heart failure.
3. The appearance of the anemia following operation coincides with a drop in basal metabolic rate.
4. The white blood cell count is decreased at low levels of basal metabolism following total thyroidectomy; the distribution of cell types remains normal; the platelet count is usually diminished. The evidence at hand indicates that the slight decrease in concentration of white blood cells usually present does not alter the response to infection.
5. Data obtained in this study support the concept that the anemia improves when the basal metabolic rate is increased by administration of thyroid; the administration of iron does not affect the blood findings.
6. At the levels of metabolism of approximately —25 per cent to —30 per cent usually maintained in patients after total thyroidectomy the blood changes are not great enough to give rise to symptoms of anemia. The anemia is not of sufficient degree to increase the work of the heart.

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# THE WORK OF THE LEFT VENTRICLE IN AORTIC INSUFFICIENCY

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Until recently there has been no unanimity of opinion among physiologists and clinicians about the amount of blood which regurgitates into the left ventricle during diastole in the presence of aortic insufficiency. Estimates have ranged from an impression that it must be a considerable amount (1, 2) to an idea which assumed a mini-

mal backward movement of blood as compared to pressure (3, 4). Wiggers, at one time chief proponent of the latter theory, has agreed since 1930 that up to 60 per cent of the tidal volume may regurgitate (5).

On a suitable schema of the circulation (6) (Figure 1) with a fixed actual stroke it can read-

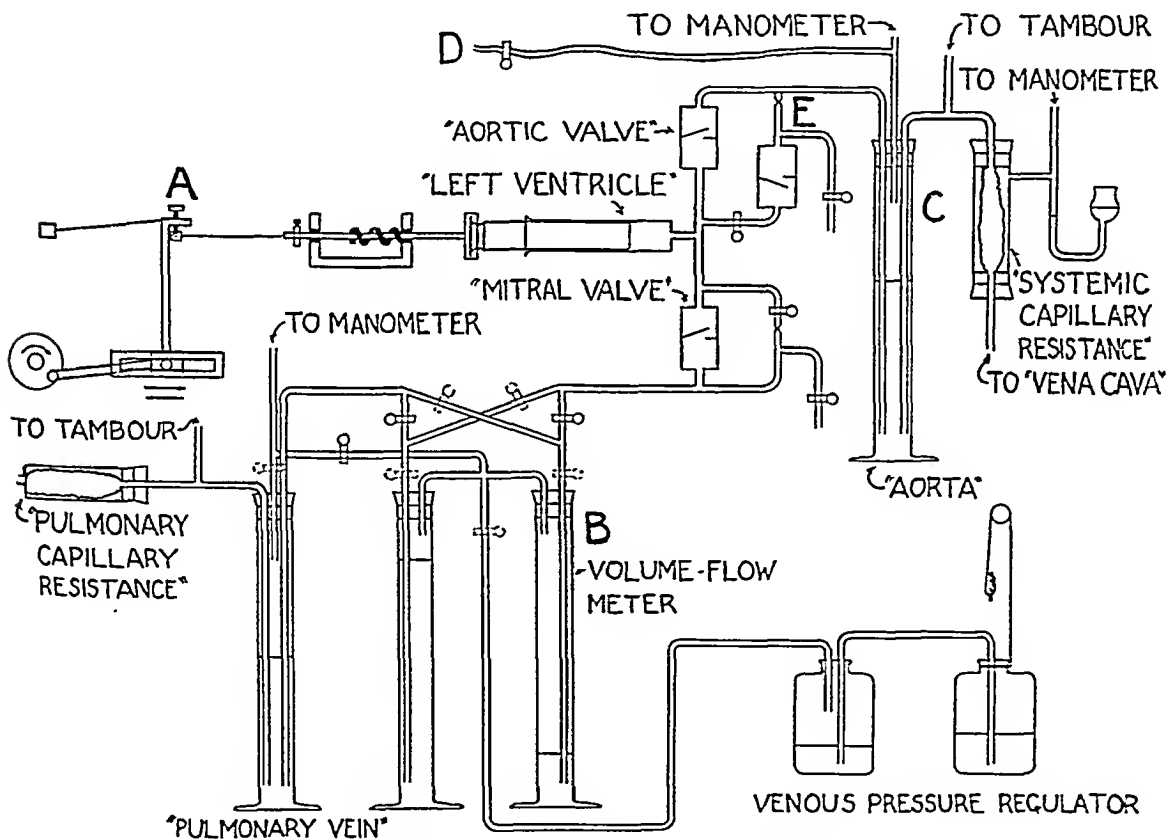


FIG. 1. DIAGRAM OF ONE-HALF OF THE SCHEMA.

Important features are: (1) Stroke volume can be minutely varied at *A* and volume flow accurately measured at *B* by Stromuhr technic. (2) Elasticity in "arteries" may be increased in two ways: introducing air in chamber, *C*, or having longer lengths of rubber tube, *D*, in the system. (3) A fixed or variable leak may be introduced in the "aortic valve" at point *E*. (4) Rate can be and was made slow enough to prevent artefacts in the readings of the mercury manometers employed. (5) Capillary resistance may be altered by varying the height of the mercury bulb to the right which controls the water pressure on the outside of the sponge-filled, thin-walled rubber tube used as the "capillary bed."

ily be demonstrated that there is progressive (Table I). There is an accompanying drop in diminution in output per beat with increase in the mean "arterial" pressure and an increase in amount of "aortic" insufficiency up to a point "pulse" pressure. Further increase in the size

TABLE I  
Effect of increasing the amount of aortic insufficiency on stroke volume in the schema

Experiment number	Output per beat	Pulse pressure	Leak	Remarks
	cc.	mm. Hg	cc.	
1	1.25	41	0	Intact valves
2	1.15	45	0.1	Aortic leak, actual stroke unchanged
3	1.06	50	0.19	Larger leak, actual stroke unchanged
4	0.97	53	0.28	Larger leak, actual stroke unchanged
5	0.83	58	0.42	Larger leak, actual stroke unchanged
6	0.55	62	0.70	Larger leak, actual stroke unchanged
7	0.42	63	0.83	Larger leak, actual stroke unchanged
8	1.20	125	2.16	Leak as in 7, stroke increased (compensation?)
9	3.41		0	Intact valves, stroke as in 8

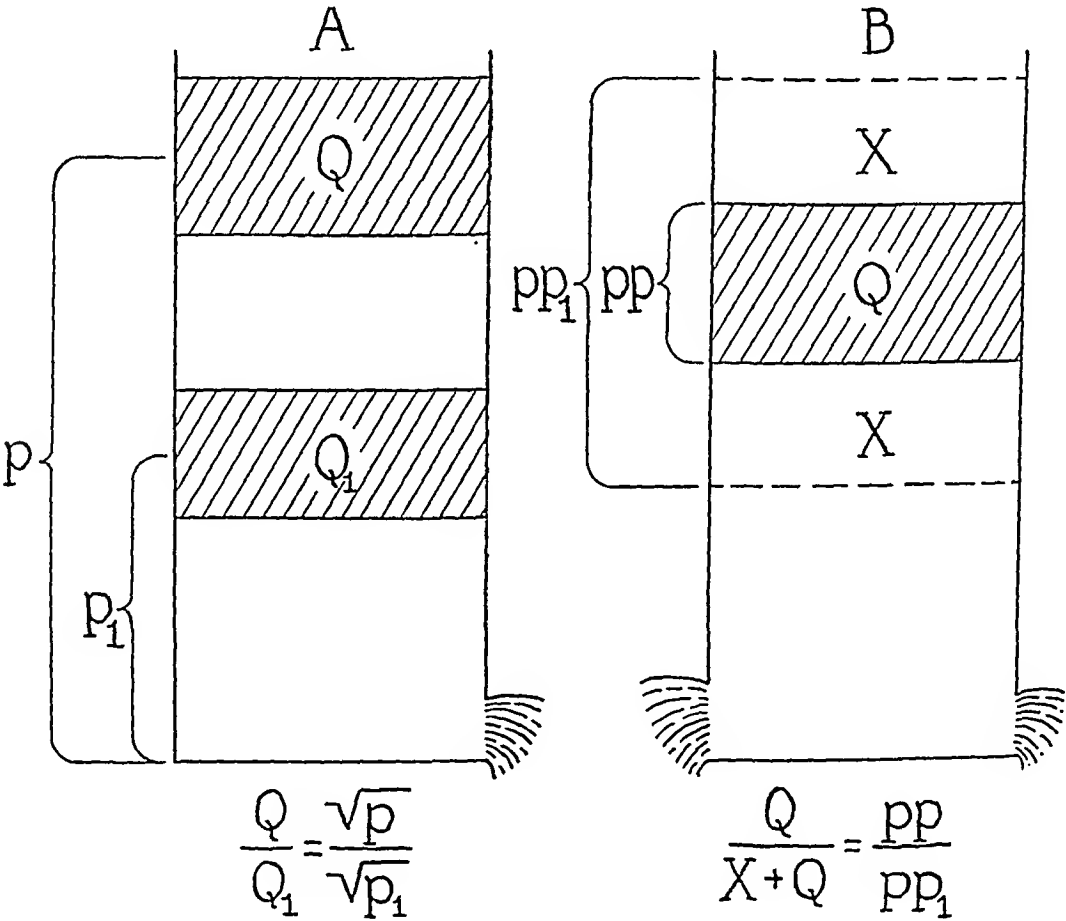


FIG. 2.

A represents a standpipe with an opening at the bottom. The amount flowing out of the opening in unit time is proportional to the square root of the mean head of pressure. B represents a standpipe with an additional opening at the bottom. The amount flowing from both openings is proportional to the amount flowing from the original opening alone as are their respective differences in height (pulse pressures). The amount flowing from one opening alone is equal to the amount flowing from that opening in the presence of an additional opening only if the mean pressures in the two conditions are equal.

of the leak has no effect upon the output or the pressure relationships unless "compensation" is attempted by increasing the actual stroke. Increasing the latter sufficiently results in a larger amount of regurgitation and a restoration of mean pressure and output per beat and a further increase in pulse pressure. A factor which may modify these results on the schema is the presence of a dominant "right ventricle" acting strongly enough to determine the volume output of the system at all times—a situation not encountered in animal or human physiology. Excluding right ventricular activity, by using one-half of the model only, a typical experiment in which peripheral resistance and elasticity in the system were kept constant has already been mentioned (Table I).

An approximate estimate of the amount of fluid which regurgitates after "compensation" has been established may be made by using only factors which are measurable in intact animals, assumptions which seem valid to me, and by employing physical formulae which are known. The calculation will be better understood by reference to Figure 2 which represents a stand-pipe with an outlet at the bottom. An old conception in hydraulics first proposed by Torricelli in 1660 (7) states that the quantity flowing out in unit time is theoretically expressed in the following equation:

$$Q = a\sqrt{2gh},$$

where  $Q$  is quantity,  $a$  is area of opening,  $g$  is the force of gravity and  $h$  is mean height or mean head of pressure. Friction, viscosity and other factors are known to influence this theoretical calculation, so that in practice a constant is necessary to make it accurate and usable in estimating any one of the factors separately. But relations between two of the factors do not require the use of the constant for accuracy.

$$\frac{Q}{Q_i} = \frac{\sqrt{h}}{\sqrt{h_i}}$$

is well established if other factors remain unchanged.

If the assumption is made that there is no change in the size of the capillaries in the basal state this conception makes possible the calculation, when the heart is normal, of systolic and

diastolic volume flow from the arteries to the capillaries by the equation:

$$\frac{Q_d}{V - Q_d} = \frac{T_d\sqrt{p_d - p_c}}{T_s\sqrt{p_s - p_c}},$$

$$Q_d = \frac{VT_d\sqrt{p_d - p_c}}{T_s\sqrt{p_s - p_c} + T_d\sqrt{p_d - p_c}}, \quad (1)$$

where  $Q_d$  is diastolic flow,  $V$  is total flow per beat,  $T_d$  and  $T_s$  are diastolic and systolic time, and  $p_d$ ,  $p_s$  and  $p_c$  are diastolic, systolic and capillary mean pressures, respectively.

With the same assumption it also permits the calculation of diastolic flow to the capillaries in the presence of an aortic leak and reduced mean diastolic arterial pressure:

$$\frac{Q_d}{Q_i} = \frac{\sqrt{p_d - p_c}}{\sqrt{p_{di} - p_c}},$$

$$Q_i = \frac{Q_d\sqrt{p_{di} - p_c}}{\sqrt{p_d - p_c}}, \quad (2)$$

where  $Q_i$  and  $p_{di}$  refer to diastolic forward flow and mean diastolic pressure in the presence of an aortic insufficiency.

The accuracy of these equations for use in the schema was tested by varying the stroke and stroke volume with a constant peripheral resistance and elasticity in the system rather than by attempting to separate systolic and diastolic flow. The increased stroke and stroke volume led to increased mean arterial pressures the square roots of which were proportional to the stroke volumes (Table II). By inspection it was also possible to show that flow from the capillaries was uniform during systole and diastole when the elas-

TABLE II

*Effect of increasing stroke volume on mean arterial pressures with constant peripheral resistance and elasticity*

Mean pressure	Capillary pressure	$\sqrt{\text{Mean pressure} - \text{capillary pressure}}$	Stroke volume	Calculated stroke volume*
mm. Hg	mm. Hg		"	"
36	21	3.88	1.42	
80	21	7.69	2.72	2.75
100	21	8.90	3.31	3.26

\* Calculated stroke volume determined by equation

$$\frac{1.42}{X} = \frac{\sqrt{p_i - p_c}}{\sqrt{p_s - p_c}}$$

ticity in the arteries was high and the pulse pressures therefore low (pulse pressure = 2 mm. Hg with a mean blood pressure of 120 mm. Hg), and that there was considerable variation in systolic and diastolic flow when the arterial system was more rigid with a considerable difference between mean systolic and mean diastolic pressure (120 mm. Hg and 80 mm. Hg, respectively).

The assumption that there is no change in the size of the capillaries between systole and diastole is probably not entirely correct and has been disputed in the case of lesions of the aortic valve (3, 4), but it is important to note that if there is any deviation it is most probably small rather than large. The quantity of diastolic flow in aortic insufficiency estimated by the use of this equation is sufficiently less than that found under normal conditions to account for the appearance of a capillary pulse in this valvular disorder.

Referring again to the analogy of the stand-pipe (Figure 2), the amount of fluid leaving the reservoir when there is an additional opening (a leak) in unit time, and the amount leaving it in the same time through only the original opening (the capillaries) are proportional as are their

respective differences in height (pulse pressures). This fact makes possible a third equation:

$$\frac{Q_d}{X + Q_l} = \frac{pp_d}{pp_l} \tag{3}$$

where  $Q_d$  and  $Q_l$  are diastolic flow without and with a leaking aortic valve (as before),  $pp_d$  and  $pp_l$  are pulse pressures without and with aortic insufficiency and  $X$  is the amount which regurgitates into the left ventricle.

Substituting for  $Q_d$  and  $Q_l$  and solving for  $X$ :

$$X = \frac{VT_d(pp_l\sqrt{p_d - p_c} - pp_d\sqrt{p_{dl} - p_c})}{pp_d(T_s\sqrt{p_s - p_c} + T_d\sqrt{p_d - p_c})} \tag{4}$$

This formula for the calculation of the amount of fluid which regurgitates into the left ventricle was tested on the model. Only a few of many observations in which the agreement was close are given (Table III).

It will be noted (Table III) that when all of the factors used in the calculation of results on the model (systolic and diastolic times were equal and cancelled out) were roughly proportionate fractions of animal and human pressures and vol-

TABLE III  
Comparison of calculated with observed leaks in the schema

Intact valves				Aortic insufficiency					Remarks
Output per beat	Pulse pressure	Mean diastolic pressure	Mean systolic pressure	Pulse pressure	Mean diastolic pressure	Observed leak "un-compensated"	Observed leak "compensated"	Calculated leak	
cc.	mm. Hg	mm. Hg	mm. Hg	mm. Hg	mm. Hg	cc.	cc.	cc.	
1.90	7	5	7	14	3		1.1	1.07	} Roughly proportionate fraction of animal output per beat and pressure
1.87	4	5	7	8	5		0.7	0.85	
1.91	7	5	7	14	2		1.2	1.20	
.93	22	81	86	44	76		0.45	0.47	} Same original condition with different sized leaks
.93	22	81	86	35	76		0.30	0.28	
.93	22	81	86	28	76		0.20	0.14	
.93	22	81	86	40	50	0.33		0.47	"Uncompensated" leak
2.71	4	96	98	12	90	0.61	1.1+	2.80	} (Extreme elasticity)
2.68	5	102	104	13	90	0.61	1.1+	2.20	
2.68	7	107	110	17	92	0.60	1.1	1.97	
2.64	12	109	113	23	94	0.57	1.1	1.30	} Varying elasticity
2.68	18	110	116	31	94	0.61	1.1-	1.06	
2.61	34	110	120	54	95	0.59	1.1-	0.86	
1.90	9	16	25	20	14	.80	1.10	1.08	} Excessive elasticity and same actual leak with increasing resistance
1.91	4	30	32	27	25	1.03	1.8+	5.43	
1.89	4	108	110	42	90	1.29	4.5+	9.23	
0.95	10	140	146	22	132		0.50	0.36	"Right ventricle" active

ume flows, the calculated leaks agreed fairly well with the observed leaks.

The widest discrepancy was observed when there was greater elasticity in the arterial system than exists in animals, especially when accompanied by an excessively high mean arterial pressure in proportion to stroke volume. The elasticity was regarded as excessive when, with large stroke volumes at high mean arterial pressures, the pulse pressures remained small. If the elasticity in the arterial reservoirs in these discordant experiments had been comparable to that in ani-

mals, such stroke volumes would result in higher pulse pressures at high mean pressures (8, 9).

The use of this formula in animal or human aortic insufficiency is of doubtful value; such complicated hydrodynamic systems are better studied empirically. But at present no technic is available with which to appraise the facts in animals or human beings. There is justification, therefore, in the use of a formula the results of using which agree as closely as does this one under natural and artificial conditions.

Theoretical considerations which may lead to incorrectly high calculations include, 1—the rôle played by the elasticity and tone of the aorta, 2—the tandem activity of the two ventricles, 3—erroneous assumptions as to what constitutes compensation, for the formula presupposes that compensation is present and assumes the restoration of usual volume flow to the tissues.

1. The part played by elasticity in the schema has already been discussed. In animals and man no serious bar to accuracy need occur unless arterial tone modifies elasticity at low mean arterial pressures in an important way. If arterial tone leads to greater rigidity at these levels and the natural limit of elasticity is approached at high mean pressures, each of these factors would result in a greater pulse pressure per unit change in volume than in the middle range of mean pressures (9). Increased rigidity, whether from arterial tone or from high mean pressures, would tend to exaggerate the gross stroke volume change including the amount of insufficiency that would occur as suggested by Equation 4 (Table IV). Calculations of smaller and moderate size leaks would not be affected, however, and those of

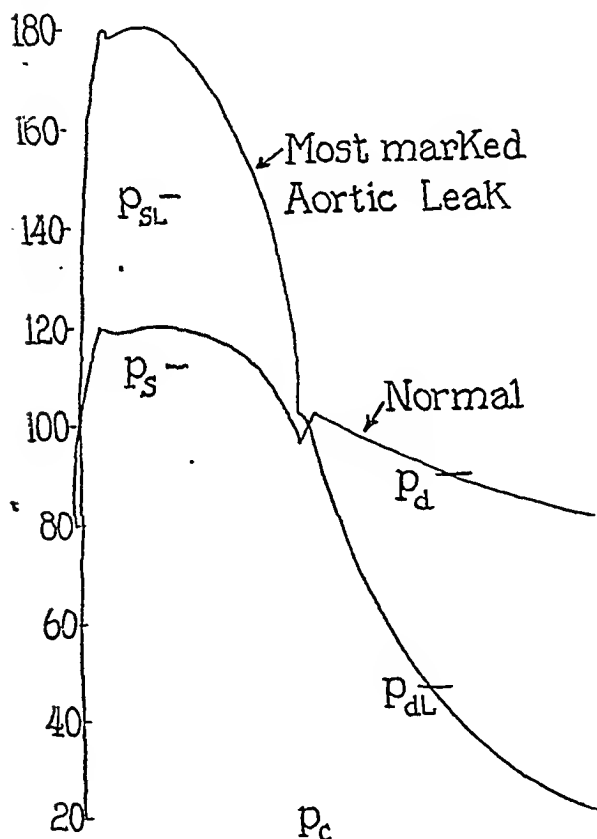


FIG. 3. HYPOTHETICAL HUMAN AORTIC PRESSURE CURVES USING DATA FROM WIGGERS (3) AND HAMILTON ET AL. (2) FOR THE SHAPES OF THE CURVES, AND TIME RELATIONS FROM ADAMS (12).

$p_{s1}$ ,  $p_s$ ,  $p_{d1}$ ,  $p_d$  and  $p_c$  represent mean systolic pressure with and without aortic insufficiency, mean diastolic pressure with and without a leak and mean capillary pressure, respectively.

TABLE IV

Calculated leaks in hypothetical human aortic insufficiency

Diastolic arterial pressure	Systolic arterial pressure, (mm. Hg)					
	130	140	150	160	170	180
mm. Hg	cc.	cc.	cc.	cc.	cc.	cc.
70	15	21	28	34	40	47
60	23	29	35	42	48	55
50	31	37	44	50	57	63
40	39	45	51	58	64	71
30	47	53	60	66	73	79
20	55	61	68	74	81	87

larger leaks only slightly. The rôle played by elasticity affects the use of the formula in a way that makes the italicized figures (Table IV), where mean arterial pressures are comparable to supposed mean arterial pressures with intact valves, probably more accurate than the remaining figures. The formula supposes the presence of normal elasticity. Normal elasticity is assumed to exist when the systolic pressure is 120 mm. Hg, the diastolic, 80 mm. Hg, the valves intact, and the output normal.

2. The tandem activity of the two ventricles affects the volume output per beat but not the calculation of the leak, unless the right ventricle is dominant, a condition which is not encountered in human beings.

3. Compensation is accomplished in aortic insufficiency by maintenance of the normal cardiac output and often by the normal output per beat (10, 11). The output per beat is assumed to be 50 cc. at a rate of 70 per minute. The leak, according to Equation 4, varies directly with the stroke volume when the valves are intact. If 50 cc. is high, the leaks will also be proportionally high. Since the cardiac output is 3.5 liters under these circumstances, a close approximation to "normal" (10, 11) has been attained.

Other factors might operate to make the calculations of the insufficiency smaller than the actuality. Among these are (a) the arbitrary use of low (20 mm. Hg) capillary pressures rather than higher arteriolar ones, and (b) the fact that diastolic intraventricular pressure is higher in animals than in the schema. Negative intraventricular pressure theoretically operates in favor of an increase in inflow from the mitral orifice rather than from the aorta through a defect in the aortic valve because the difference in pressures in the left auricle and in the aorta becomes relatively less as intraventricular pressure becomes more negative. (c) A third factor is the fact that systole is longer and diastole correspondingly shorter in electrocardiograms than when measured in mechanical records (0.38 second for systolic time and 0.47 second for diastolic time (12) were used).

The figures for the amounts which flow back in human aortic insufficiencies (Table IV) are compatible with experience under many different conditions in the model and are reasonable from

the clinical and pathological point of view. The largest calculated leak would entail a gross stroke volume output of 137 cc. (87 cc. regurgitated (Table IV) plus 50 cc. output per beat) which would seem possible when the enlargement of the left ventricular chamber known to be common in severe examples of this valvular disease is considered.

The amount of leak plus the net stroke volume makes possible the calculation of work done by the left ventricle. Hamilton has shown that the velocity factor of work done by the left ventricle is an appreciable amount and is demonstrable in animals as an elevation of intraventricular pressure above aortic pressure (13). The increased gross stroke volume in aortic insufficiency increases this velocity factor. If this factor is disregarded the following simple formula may be used:

$$\text{Work (H.W.U.)} = (V + X)p_{sl}$$

where H.W.U. equals an arbitrary work unit,<sup>1</sup>  $V$  equals net stroke volume,  $X$  equals the amount of leak and  $p_{sl}$  equals mean systolic arterial pressure in presence of a leak (derived from Wiggers' curves). The calculations of work done by the left ventricle with various size leaks show that the work increases with increase in the size of the valvular defect (Table V).

An attempt has been made (10, 14) to utilize left ventricular work and heart size in proposing an index of the function of cardiac muscle. While this is a promising approach it should be noted that when data for cases of aortic insufficiency with presumed impending myocardial insufficiency are recalculated on the basis of this formula the relationship is altered so that the cases fall well within the normal range. Thus Case 24 (14) has a calculated grammer per beat of 123 instead of 43, Case 57 (10), 81 instead of 34 and Case 201 (10), 156 instead of 52.5. These calculations represent changes in the values

<sup>1</sup> A work unit is employed which avoids multiplication of the results by factors representing absolute work units. A new and simple work unit for clinical purposes is legitimate and desirable. The use of absolute work units creates an illusion of accuracy not justified by the facts in this and other calculations of cardiac work. The work unit, which is 1 cc. of blood times 1 mm. Hg pressure, is here designated as a "heart work unit" (H.W.U.).

TABLE V

*Calculated basal work per beat of the left ventricle in hypothetical human aortic insufficiency\**

Diastolic arterial pressure	Systolic arterial pressures, (mm. Hg)											
	130		140		150		160		170		180	
	Work in H.W.U.†	Per cent of normal	Work in H.W.U.†	Per cent of normal	Work in H.W.U.†	Per cent of normal	Work in H.W.U.†	Per cent of normal	Work in H.W.U.†	Per cent of normal	Work in H.W.U.†	Per cent of normal
mm. Hg												
70	7570	135	8680	155	9930	177	11250	201	12670	226	14120	252
60	8500	152	9650	172	10950	196	12320	220	13780	246	15280	273
50	9449	169	10620	190	11990	214	13390	239	14900	266	16470	294
40	10380	185	11600	207	13000	232	14480	258	16020	286	17620	315
30	11320	203	12600	225	14030	251	15590	278	17150	306	18820	337
20	12240	219	13600	243	15050	269	16650	298	18300	327	20020	358

\* Basal work with intact valves =  $50 \times 112 = 5600$  H.W.U.

† For explanation see footnote 1, page 648.

of the systolic mean pressures based on reconstructed curves mentioned before as well as changes in the gross stroke volume.

The heart, as opposed to the left ventricle only, must do increased work in other valvular lesions such as mitral stenosis, a fact readily shown on the schema but not calculable in living man. Increased work would alter the ratio of heart work to heart size for all valvular lesions and make necessary a revision of the conception of this ratio as an index of function. It may well be that the ratio is important in actual heart failure but reliable figures on output are difficult to obtain at present in that state. The ratio may also be important as a means of estimating cardiac work in patients with compensated valvular lesions of a type which does not permit the direct calculation of work.

Since no technic is at present available for estimating the immediate or future capacities of the left ventricle in a given case to do work, these figures cannot be used except to give a conception of the enormous increase in work *continuously* necessary in this valvular lesion.

#### SUMMARY AND CONCLUSIONS

1. Increasing amounts of fluid regurgitate into the "left ventricle" of a schema of the circulation in the presence of increasingly large "aortic insufficiencies."

2. A formula for the calculation of these amounts has been constructed using peripheral findings measurable in man, assumptions which

seem valid and are compatible with known physical laws.

3. The formula gives comparable results to the leaks measured in the schema when elasticity, stroke volumes and mean pressures are proportionate fractions of their values in animals and man.

4. The formula applied to hypothetical human aortic insufficiencies of various sizes gives approximations of the amount of regurgitation which vary from 15 cc. to 87 cc. per beat with an assumed net stroke volume of 50 cc. and a rate of 70 per minute. A discussion of the accuracy of the approximations and the factors affecting it reveals no important large source of error.

5. The work of the left ventricle in these hypothetical cases is calculated from the gross stroke and mean systolic arterial pressure omitting the probably important velocity factor and still amounting to 135 to 358 per cent of basal work with intact valves.

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# THE PERIPHERAL BLOOD FLOW IN SURGICAL SHOCK

## THE REDUCTION IN CIRCULATION THROUGH THE HAND RESULTING FROM PAIN, FEAR, COLD, AND ASPHYXIA, WITH QUANTITATIVE MEASUREMENTS OF THE VOLUME FLOW OF BLOOD IN CLINICAL CASES OF SURGICAL SHOCK

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Patients in surgical shock present clinical evidence of a diminished peripheral circulation. The extremities are cold, the pulse is feeble, and the veins are empty of blood. An increase in the peripheral vascular resistance in experimental shock has been demonstrated by Erlanger, Gesell and Gasser (1). Quantitative measurements, however, on the reduction in circulation in human cases of shock have not been made. These studies were therefore undertaken to learn the extent of impairment in the distribution of blood to the peripheral tissues. In addition, the effect of traumatic stimuli on the volume flow of blood in normal individuals was studied under controlled conditions.

### METHODS

The subjects were patients and medical students at the Massachusetts General Hospital. The volume flow of blood through the hand at controlled temperatures was measured by the plethysmographic method previously described (2). In the observations on surgical shock the blood flow was determined with the hand at different temperatures. Then, at a constant temperature, the reactive hyperemia was measured 10 seconds after release of a tourniquet which had occluded the arterial inflow for 5 minutes. Samples of arterial and venous blood were taken when feasible for oxygen content, oxygen capacity and carbon dioxide content. The oxygen content, oxygen capacity and carbon dioxide content were determined in duplicate by the method of Van Slyke and Neill (3). Studies were carried out in the laboratory, in the operating amphitheatre, and on the wards. Notes were taken on the room temperature, clinical condition of the patient, and any incidental stimuli.

The reactions of patients and students to the traumatic stimuli—cold, fear, pain and asphyxia—were studied.

When the effect of cold was studied, the subject lay scantily clad on a bed for one-half hour with the temperature of the hand stabilized at 30° C. The window was then opened and the room temperature allowed to fall to between 15° and 18° C. After a short period of

cold, the window was closed and the room temperature allowed to rise.

Fear was produced by suggesting to the patient some painful or repulsive procedure which was to be carried out. The subject was unaware that his reactions were being noted.

Pain was precipitated, with the consent of the subject, through faradic stimulation of the skin and other measures.

Partial asphyxia was brought about by having the subject breathe the gas with a reduced concentration of oxygen (between 7.1 and 15 volumes per cent) delivered from a Tissot spirometer. Rebreathing was avoided. After the experiment had been concluded, the oxygen content of the gas mixture was analyzed by the Haldane apparatus. In the experiments in which asphyxia was employed the temperature of the bath in which the hand was immersed was maintained between 32° and 34° C. The subject, after resting for half an hour, was connected to the spirometer tubing, but breathed room air for a period of 10 to 15 minutes while determinations of blood flow were being made. After a stable flow had been recorded during this control period, the valve was turned to the spirometer. Precautions were taken to prevent the subject from being aware of the change in gas mixture. The period of partial asphyxia lasted for 10 to 40 minutes. During this time, observations on blood flow were made at frequent intervals. The valve was then turned to room air, and a second series of control observations was made. Between 4 and 9 measurements of the volume flow of blood were taken at each determination during the experiment. On the average, 8 groups of determinations were made during the period of asphyxia, while 9 groups of determinations were made during the control periods. The volume of blood flows listed in Table I are each, therefore, the average of approximately 60 measurements.

### A. TRAUMATIC STIMULI

#### *Results*

Cold uniformly reduced the volume flow of blood even though the hand was maintained at constant temperature. Figure 1 illustrates the reduction in circulation when the room temperature was lowered from 22° to 16° C. Similar results

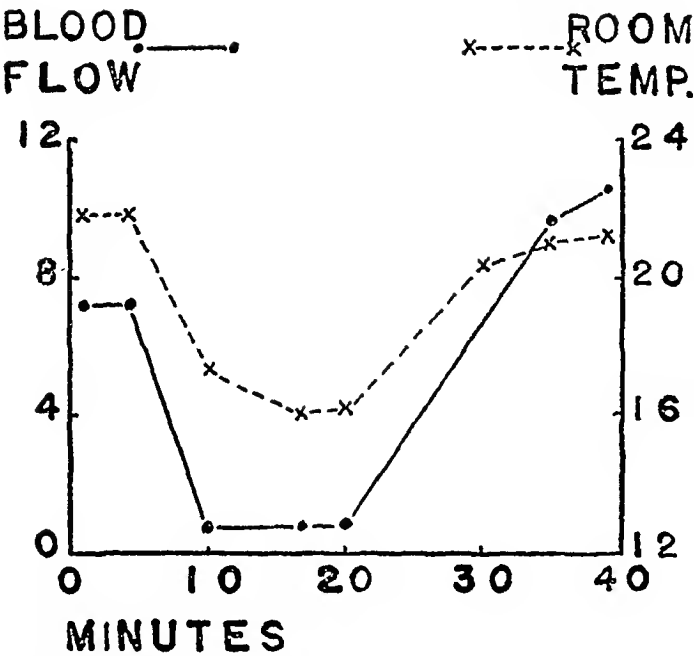


FIG. 1. EFFECT OF LOWERING ROOM TEMPERATURE ON VOLUME FLOW OF BLOOD THROUGH THE HAND MAINTAINED AT CONSTANT TEMPERATURE ( $32.4 \pm .3^{\circ} \text{C.}$ ).

Ordinates: cubic centimeters of blood flow per 100 cc. hand volume per minute and room temperature, degrees Centigrade. Abscissae: time, minutes. Solid line: blood flow; interrupted line: room temperature.

were obtained in each experiment on 3 different individuals.

The effect of pain produced by the inflation of a balloon which had been inserted, with the patient's consent, into an ileostomy opening<sup>1</sup> is illustrated in Figure 2. The balloon was inserted at A. Manipulation at B and C did not alter the blood flow. Inflation of the balloon inside the ileostomy (D) now caused the patient to complain of cramp-like pain, and within a few minutes he became nauseated. The rate of blood flow diminished sharply and continued to be depressed as long as the cramps persisted. It returned promptly to the original level when the balloon was deflated. Eight experiments with various types of pain gave essentially similar results.

Figure 3 demonstrates the effect of apprehen-

<sup>1</sup>The patient had had an ileostomy performed 18 months previously for ulcerative colitis. The colon had been removed 6 months later. Present observations were made, with the consent of the patient, 2 weeks after a minor operation.

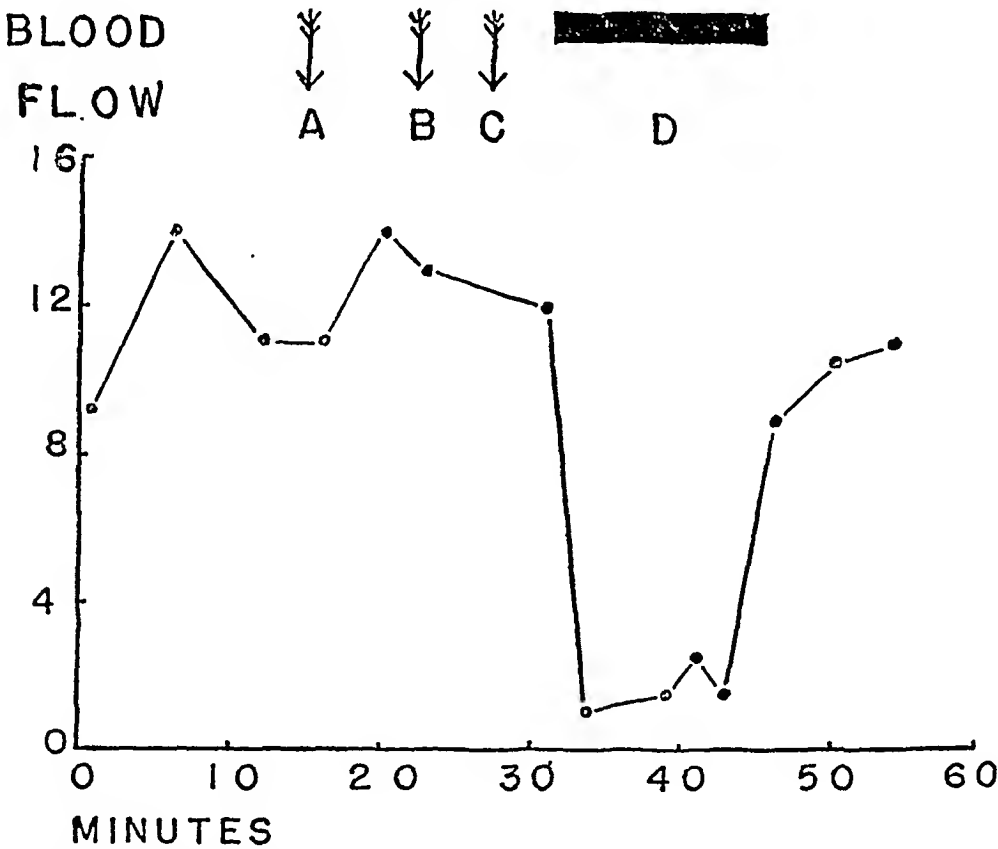


FIG. 2. EFFECT OF PAIN, PRODUCED BY INFLATION OF A BALLOON IN THE ILEUM, ON THE VOLUME FLOW OF BLOOD THROUGH THE HAND MAINTAINED AT CONSTANT TEMPERATURE ( $31.6 \pm .4^{\circ} \text{C.}$ ).

Ordinates: cubic centimeters of blood flow per 100 cc. hand volume per minute. Abscissae: time, minutes. A row A indicates the insertion of the balloon into the ileum. The balloon was manipulated at arrows B and C. The broad black line, D, indicates the inflation of the balloon.

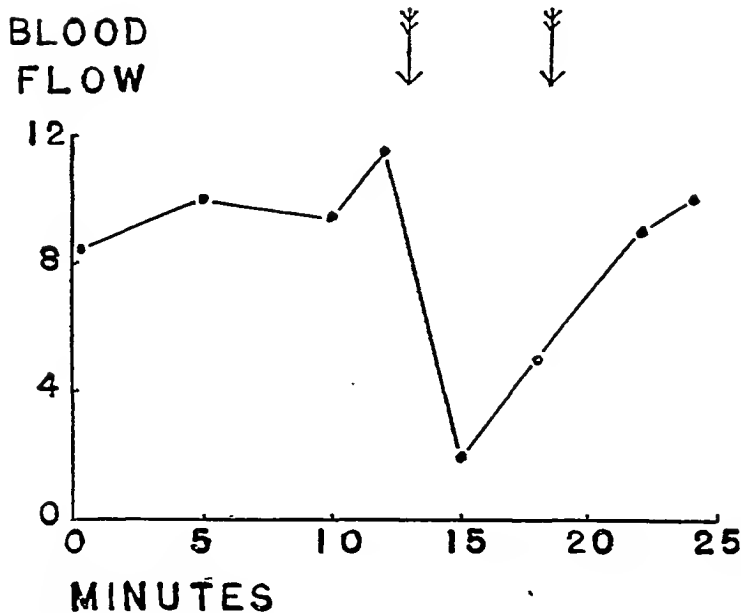


FIG. 3. EFFECT OF APPREHENSION ON VOLUME FLOW OF BLOOD THROUGH THE HAND, MAINTAINED AT CONSTANT TEMPERATURE.

Ordinates: cubic centimeters of blood flow per 100 cc. hand volume per minute. Abscissae: time, minutes. At the first arrow, the subject was informed that a balloon was to be inserted into her colostomy. The balloon was actually inserted at the second arrow.

sion on the rate of blood flow. The subject<sup>2</sup> was accustomed to the apparatus by previous experiments. As soon as the rate of flow became relatively stable, the subject was informed that a balloon was to be inserted into her colostomy. She made no comment, but her facial expression revealed apprehension and repugnance as the balloon was being prepared and the dressing removed from the colostomy. The volume flow of blood declined from 10 to 2 cc. per minute within 2 minutes (Figure 3, first arrow). The return of the circulation was not affected by the presence of the balloon in the colon (second arrow). A decrease in blood flow from emotional trauma so frequently accompanied experiments designed for other purposes that it was eliminated only with great difficulty. Not only fear and apprehension but embarrassment, disgust, anxiety and annoyance reduced the rate of blood flow. Loud noises and interruptions were carefully avoided, and the patient was even cautioned against spontaneous thoughts of a high emotional content.

<sup>2</sup> The subject had had a colostomy performed 3 weeks previously for relief of a rectal stricture.

Partial asphyxia<sup>3</sup> generally produced an increase in the volume flow of blood, although frequent reductions in circulation were noted. The experimental data are listed in Table I. No correlation of the direction of the change with the concentration of oxygen was observed. In Experiment 6, the blood flow increased 37 per cent when the oxygen was reduced to 7.1 volumes per cent. It decreased 38 per cent in Experiment 15 with a similar concentration (7.3 volumes per cent). In the same individual (J. C. S.) a concentration of 13.2 volumes per cent was associated with an increase of 45 per cent on one occasion (Experiment 2), while on another occasion (Experiment 14), with practically the same amount of oxygen, the blood flow decreased by 12 per cent.

Since it is recognized that asphyxia stimulates the sympathetic nervous system (4), experiments were conducted on patients in whom the vasoconstrictors to the upper extremity had been previously interrupted because of Raynaud's disease.

<sup>3</sup> The authors wish to express their appreciation to Dr. D. B. Dill of the Harvard Fatigue Laboratory for his assistance in the experiments on asphyxia.

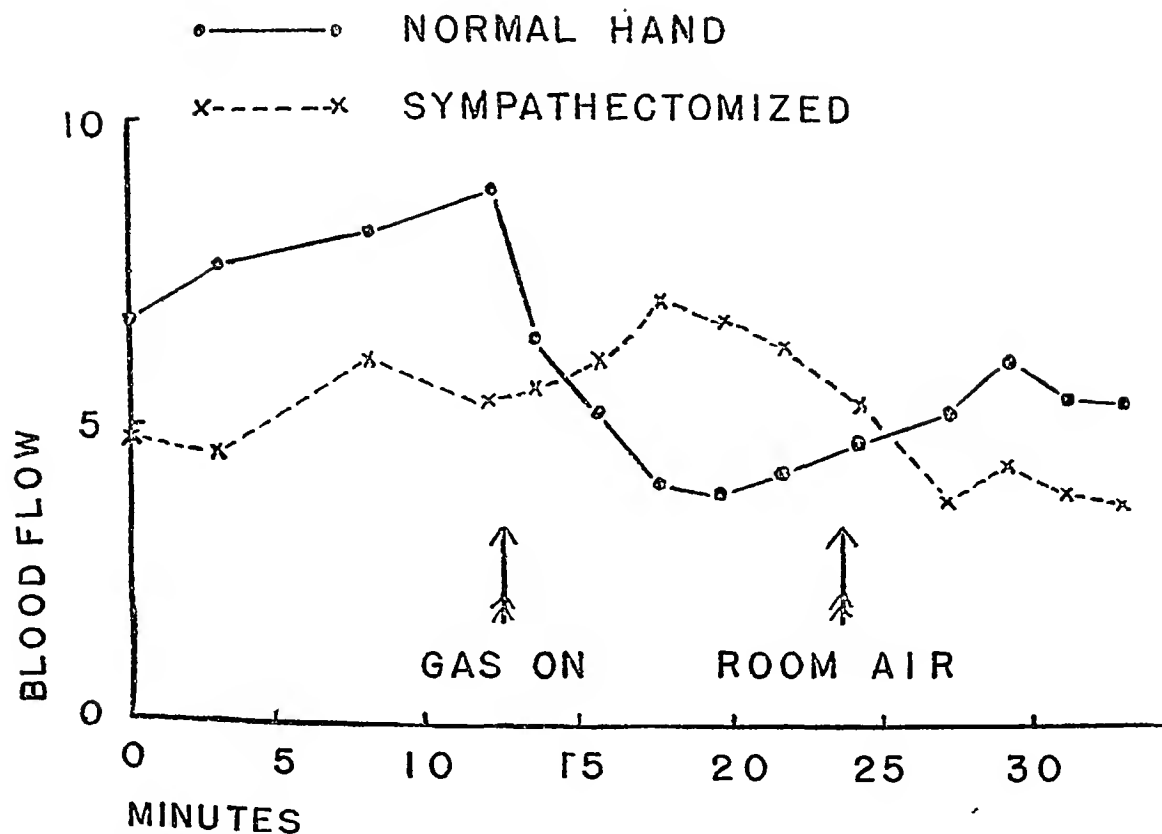


FIG. 4. EFFECT OF ASPHYXIA ON THE VOLUME FLOW OF BLOOD THROUGH THE HANDS MAINTAINED AT CONSTANT TEMPERATURE.

Solid line: left hand, normal ( $33.0 \pm .4^\circ \text{C.}$ ). Interrupted line: right hand sympathectomized ( $33.4 \pm .6^\circ \text{C.}$ ). Ordinates: cubic centimeters of blood flow per 100 cc. hand volume per minute. Abscissae: time, minutes.

In each experiment (see Table I), asphyxia brought about an increase in the blood flow through the sympathectomized hand. In two patients, in whom only one extremity had been sympathectomized, the effect of asphyxia on the circulation was determined in both hands simultaneously. Figure 4 illustrates the reaction which one of the patients presented. With asphyxia, induced by breathing a gas mixture containing 11.88 per cent oxygen, the blood flow through the normal hand declined abruptly, while at the same time it increased on the sympathectomized side. The gradual decline of blood flow through the hand in which the vasomotor nerves had been interrupted may have been the result of adrenal secretion (5). In the other patient (Table I, Experiment 19) the blood flow increased by 66 per cent on the sympathectomized side, while only a slight change was noted on the side with intact vasomotor innervation.

#### B. SURGICAL SHOCK

Before attempting to determine whether or not the peripheral circulation in shock is reduced, it is necessary to define a normal blood flow.

There are many factors which influence the volume flow of blood through the hand in the normal individual. The effect of cold, pain, fear and asphyxia have been shown above. In a previous communication, the part played by temperature in controlling the circulation has been discussed (2). The basal metabolic rate also has an important effect (6). In addition to the various factors which are recognized, there may be many others, physiological or pathological, which have not been elucidated. Without recognizing *all* the factors, it is impossible adequately to control the variables so that basal conditions can be maintained. We were, therefore, forced to a numerical comparison of the average rates of blood flow in normal individuals and patients in shock under a given set of

TABLE I  
Effect of asphyxia on volume flow of blood

Experiment number	Name	Age	Sex	O <sub>2</sub> concentration	Control periods				Asphyxia periods				Per cent change in blood flow	Remarks
					Blood flow	Respiration	Pulse	Blood pressure	Blood flow	Respiration	Pulse	Blood pressure		
		years		volumes per cent	cc. per minute per 100 cc. hand volume	per minute	per minute	mm. Hg	cc. per minute per 100 cc. hand volume	per minute	per minute	mm. Hg		
NORMAL														
1	J.C.S.	25	M.	15.0	12.1	13	56		13.2	8	60		+9	Respiration deep.
2	J.C.S.	25	M.	13.2	6.1	12	50	110/68	8.9	8	55	100/64	+45	Lips dusky. Respiration deep with inspiratory pause.
3	J.C.S.	25	M.	10.0	9.2				10.9				+18	
4	D.B.D.	44	M.	11.5	6.9	18	72	120/70	8.7	11	84	122/76	+26	Arterial oxygen saturation 59 per cent.
5	D.B.D.	44	M.	10.7	3.2	16	56	104/70	3.9	7	70	108/68	+22	Moderate cyanosis of lips
6	D.B.D.	44	M.	7.1	2.4	14	76	120/80	3.3	20	100	130/70	+37	Lips and nail beds cyanotic. Unconscious. Convulsive movements.
7	F.C.	22	M.	10.7	12.5	20	60	118/76	14.0	18	80	124/72	+11	Lips and fingers cyanotic.
8	F.C.	22	M.	10.7	14.0	18	60	122/76	19.6	18	84	128/68	+40	Lips and fingers cyanotic
9	J.L.	21	M.	11.6	1.2	20	90	130/80	4.4	20	108	134/78	+260	Arterial oxygen saturation 51 per cent. Lips and fingers slightly cyanotic.
10	J.L.S.	33	M.	13.0	6.2	14	66	123/64	6.2	12	80	134/60		
11	J.L.S.	33	M.	13.5	2.8	16	72	130/68	1.5	14	78	130/85	-46	
12	J.L.S.	33	M.	10.2	3.7				2.9				-21	
13	J.L.S.	33	M.	10.0	7.6				4.6				-39	
14	J.C.S.	25	M.	13.1	5.7	12	46	100/62	5.0	10	60	110/64	-12	Lips dusky. Slightly dizzy.
15	H.D.	21	M.	7.3	8.5	16		130/50	5.1	20			-35	Arterial oxygen saturation 46 per cent. Lips cyanotic. Convulsive movements.
SYMPATHECTOMIZED														
16	P.R.	24	F.	15.0	6.1	18	60	110/54	9.3	10	72	105/52	+52	Slight dizziness.
17	P.R.	24	F.	12.5	5.7	16	60	110/62	9.1	22	60	110/60	+60	Nail beds dusky. Dizzy. Nauseated.
18	P.R.	24	F.	11.7	6.1				8.3				+36	Slight cyanosis.
19	M.E.B.	23	F.	8.17	1.7	(Normal hand)			1.9				+12	Exact gas mixture not known because of faulty nose clip.
					7.7	(Sympathectomized)			12.8				+65	
20	E.L.	22	F.	11.9	6.3	(Normal hand)			4.9				-22	Cyanosis of lips.
					4.5	(Sympathectomized)			6.0				+33	

conditions, with the realization that many variables might be operative.

An additional method of ascertaining whether or not the peripheral circulation is reduced in shock is to evaluate one of the functions of the blood flow, e.g., the nutrition of the tissues. The volume of blood flow through the hand at any specific temperature is determined, within certain limits, first, by the metabolic needs of the hand, and secondly, by the needs of the body for thermal regulation (2). It is impossible to separate the

total flow into a part which is utilized in heat loss and another portion used for tissue nutrition. In the sympathectomized hand, however, the heat-regulating mechanism is absent. The entire blood flow is probably related to the tissue needs (2). By noting the rate of blood flow through the sympathectomized hand at a specific temperature, it is possible to state what the "normal" circulation from the standpoint of tissue nutrition at that temperature should be. Additional information in relation to the nutrition of the body tissues

can also be gained by noting the differences between the oxygen content of the arterial and the venous blood.

Another index of the state of tissue nutrition is afforded by the phenomenon of reactive hyperemia. It has previously been shown (2) that the blood flow debt, created through obstruction of the arterial inflow, is exactly repaid during the period of reactive hyperemia. The studies were made on the sympathectomized hand in which the normal blood flow presumably just satisfied the tissue demands. When the tissue needs were increased through deprivation of arterial blood, the increase in blood flow after release of the tourniquet not only indicated the greater tissue demand, but also demonstrated the ability on the part of the circulation to reimburse the tissues for this blood flow debt. Reactive hyperemia was therefore studied in patients in

order to learn whether or not it was possible for the circulation in surgical shock to meet increased demands of the tissues.

### Results

The peripheral circulation in the normal individual is represented in Figure 5. The patient, N. G. (Case 1, Table II) was a 47 year old Armenian clerk, who was studied in the laboratory 2 days after an operation, under local anesthesia, for ligation and injection of varicose veins. He was ambulatory and suffering only mild inconvenience. His mouth temperature was 99.1° F., and his pulse, respiration and blood pressure were normal. As the temperature of the hand was raised, the rate of blood flow increased. When the temperature of the hand was at 37.4° C., the volume flow of blood reached 18 cc. per 100 cc.

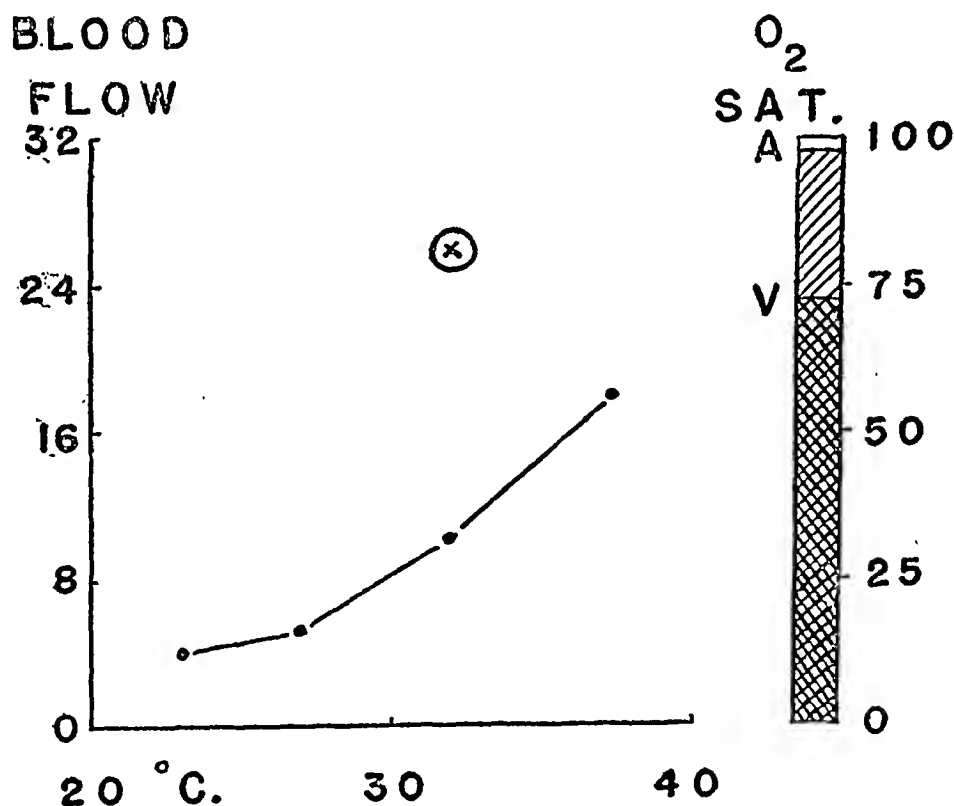


FIG. 5. NORMAL CONTROL.

Solid line: effect of increasing the temperature of the hand on the volume flow of blood. Ordinates: cubic centimeters of blood flow per 100 cc. hand volume per minute. Abscissae: temperature of bath in which hand was immersed. The cross surrounded by the circle indicates the flow 10 seconds after release of a tourniquet which had occluded the circulation at 31.8° C. for 5 minutes. Column at right: oxygen saturation of A, the arterial, and V, the venous blood. The samples of arterial and venous blood were taken at the conclusion of the observations.

hand volume per minute. The results in 2 other normal patients (Cases 2 and 3) are summarized in Table II. The average blood flow at 37° C. in 26 normal patients (data previously obtained) was 9.8 cc. per 100 cc. hand volume per minute. The values ranged from 6 to 26 cc. per minute. In 9 sympathectomized hands, in which the blood flow just equalled the nutritional needs (*vide supra*), the average flow at 37° C. was 7.8 cc. per 100 cc. hand volume per minute. The individual readings varied only between 5 and 10 cc. per minute. From these data, the normal blood flow at 37° C. was considered, for purposes of comparison, to be above 7 cc. per minute, per 100 cc. hand volume.

The cross surrounded by the circle in Figure 5, indicates the volume flow of blood, 26 cc. per 100 cc. hand volume per minute, determined 10 seconds after release of a tourniquet which had

occluded the circulation for 5 minutes at 31.8° C. When the tissue needs were increased by temporary deprivation of blood flow, prompt and effective compensation followed release of the obstruction. Similar results were obtained in the normal controls (Cases 2 and 3 and confirm the data previously obtained in sympathectomized hands (2)).

The oxygen saturation of the arterial blood (Figure 5, A) was 99 per cent. The saturation of the venous blood, 73 per cent (Figure 5, V), indicated that the circulation was quite adequate. The normal range of the venous oxygen saturation is between 60 and 85 per cent (7).

In comparison to this normal control, Figure 6 represents the circulation in a typical case of surgical shock. The patient, M. T. D., was an Irish housewife of 54, who was suffering from intestinal obstruction due to carcinoma of the sig-

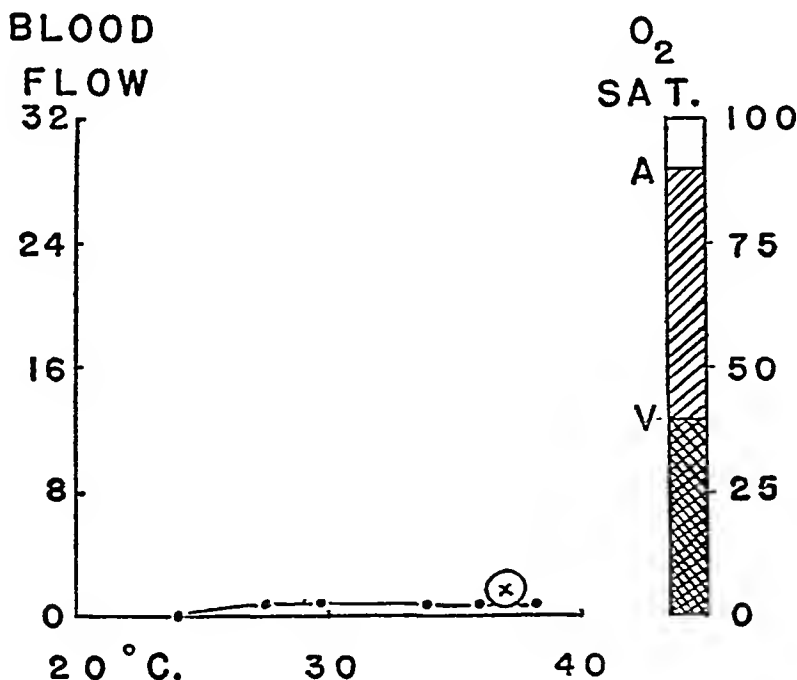


FIG. 6. SURGICAL SHOCK.

Solid line: effect of increasing the temperature of the hand on the volume flow of blood. Ordinates: cubic centimeters of blood flow per 100 cc. hand volume per minute. Abscissae: temperature of bath in which hand was immersed. The cross surrounded by the circle indicates the flow 10 seconds after release of a tourniquet which had occluded the circulation at 36.8° C. for 5 minutes. Column at right: oxygen saturation of A, the arterial, and V, the venous blood. The samples of arterial and venous blood were taken at the conclusion of the observations.



TABLE II  
Effect of general condition on volume flow of blood

Case number	Name	General condition	Sex	Age	Clinical diagnosis	Room temperature	Temperature	Pulse	Respiration	Blood pressure	Blood flow at 37° C.	Blood flow during reactive hyperemia	O <sub>2</sub> saturation		CO <sub>2</sub> content	Comment
													Arterial	Venous		
				years		° C.	° F.			mm. Hg	cc. per minute	cc. per minute	per cent	per cent	volumes per cent	
1	N. G.	Good	M.	47	Varicose veins 2 days after ligation and injection	22.1	99.1	80	22	110/80	18.0	26	99.0	73.0	43.0	Normal control.
2	W. McD.	Good	M.	48	Cervical abscess 5 days after incision and drainage	22.1	99.1	86	20		7.0	13.2	87.0	67.0		Normal control.
3	M. C.	Good	M.	23	Inguinal hernia preoperative	23.8	98.6	66	20	130/80	14.0	20.0	94.0	79.0	44.0	Normal control.
4	M. O.	Poor	F.	46	Volvulus of small intestine	21.5	101.6	138 116	20 16	78/60 140/70	1.6 7.6	3.6 20.0		41.2	53.0	Severe shock. Immediately after 500 cc. 6 per cent acacia.
5	A. M.	Poor	F.	45	Pelvic sarcoma 7 days after exploratory laparotomy	21.7	103.2	135	24	95/70	3.5			32.5	35.1	Died next day.
6	M. T. D.	Poor	F.	54	Carcinoma of sigmoid, intestinal obstruction, pelvic peritonitis	24.3	101.0	126	30	80/?	.8	1.0	90.0	41.5	53.0	4 hours after cecostomy. Died 5 hours later.
7	K. S.	Poor	F.	66	Diverticulitis of sigmoid 3 days after cecostomy	22.0	100.0	80	30	135/44	5.7	6.5	96.7	77.0	57.2	Barely recovered after 12 weeks. Gangrene of wound. Pressure necrosis of buttocks
8	J. M.	Poor	M.	53	Carcinoma of stomach, exploratory laparotomy	24.5 25.4 24.4	101.0 100.6	120 120 140	22 28 22	100/62 120/90 86/56	8.0 1.3 4.0	21.0 2.2 9.0	96.0 85.2	32.6 58.7	45.5 32.5	1 day preoperative. During operation. 4 hours postoperative. 3 days postoperative. Final recovery.
9	A. L. S.	Fair	M.	62	Carcinoma of sigmoid, cecostomy followed by combined abdominoperineal resection of rectum	22.3 24.9 25.2	98.6 100.0 98.6	80 80 84	20 20 20	124/76 120/80 120/70	3.8 8.3 9.5	9.0 37.0 30.0	98.6 35.7 90.8	31.1 35.7 80.0	63.5 64.0 48.5	10 days after cecostomy. 16 days after cecostomy. 24 days after cecostomy. Combined abdominoperineal resection of rectum 7 days later. Recovery.
10	J. B.	Poor	M.	47	Carcinoma of bile ducts. Before exploratory laparotomy	22.7	98.1	60	16	140/80	6.5	7.1	98.0	72.0	49.0	Poor general condition. Exploratory laparotomy sent blood pressure to 80/50. Recovery.
11	A. B.	Fair	M.	55	Tabs, luetic aortitis, arsenic poisoning	22.5	98.0	100	20	80/40	13.0		96.0	80.0	37.9	Recovery from low blood pressure. Sudden collapse 24 hours later.

moid. Perforation with pelvic peritonitis had occurred. She was studied on the ward, 4 hours after a cecostomy had been performed under local anesthesia. At this time her temperature was 101° F., her pulse 126, respiration 30, and blood pressure 80/?. Her skin was cold and clammy. Her tongue was dry. She was restless and kept crying for water. Her face was grey and drenched in sweat. Her blood flow failed to increase when the temperature of the hand was raised. Even at 37° C. the maximum flow was only 0.8 cc. per 100 cc. hand volume per minute.

After release of a tourniquet, applied for 5 minutes at that temperature, the reactive hyperemia amounted to only 1.0 cc. Although the oxygen saturation of her arterial blood was close to normal (90 per cent), the venous blood, taken at the conclusion of the observations, was only 41.5 per cent saturated with oxygen. In spite of a second transfusion, she failed to rally and died 5 hours later.

In Table II are listed the significant findings in 5 additional patients in poor condition (Cases 4, 5, 7, 8, and 9). In no case was the blood flow

higher than 6.5 cc. per minute at 37° C., and the reactive hyperemia was markedly reduced. The oxygen saturation of the venous blood was low in every patient but one (Case 7). In 2 of the patients the process of recovery was followed (Cases 8 and 9). As the clinical condition of the patient improved, the volume flow of blood increased. The reactive hyperemia and the oxygen content of the venous blood indicated that the circulation had become more adequate to care for the tissue needs.

Precisely which of the traumatic stimuli was responsible for the reduction in circulation in any particular patient, it was difficult to say. A certain degree of apprehension was probably associated merely with the patient's presence in the hospital. Cold was generally avoided by adequate protection. The effect of pain, however, was frequently observed. Even though the patient's consciousness might have been dulled by a general anesthetic, the physiological effects of pain could be discerned. The reaction of the circulation through the hand to manual dilatation of the pyloric sphincter is illustrated in Figure 7. The

patient, J. P. F., male, age 41, was being operated upon for a gastro-jejuno-colic fistula under avertin (100 mgm. per kilo) and ether anesthesia. At the time indicated by the arrow the pylorus was dilated by the finger of the surgeon, inserted through an opening in the wall of the stomach. The rate of blood flow, which was originally rapid from the ether anesthesia, dropped to 2.4 cc. per minute. Traction on the mesentery and manipulation of the abdominal contents produced comparable effects.

The effect of asphyxia is shown in Figure 8. This patient was markedly emaciated from pulmonary tuberculosis. His right lung had been collapsed by a thoracoplasty done in 3 stages. After the third operation the tuberculous process had extended to the opposite side with extensive involvement of the left lung. Although he was unaware of any change in the mixture of gas which he was receiving, reduction of his oxygen intake caused a marked decrease in his peripheral circulation.

A reduced effective blood volume, either from hemorrhage, transudation, exudation or dehy-

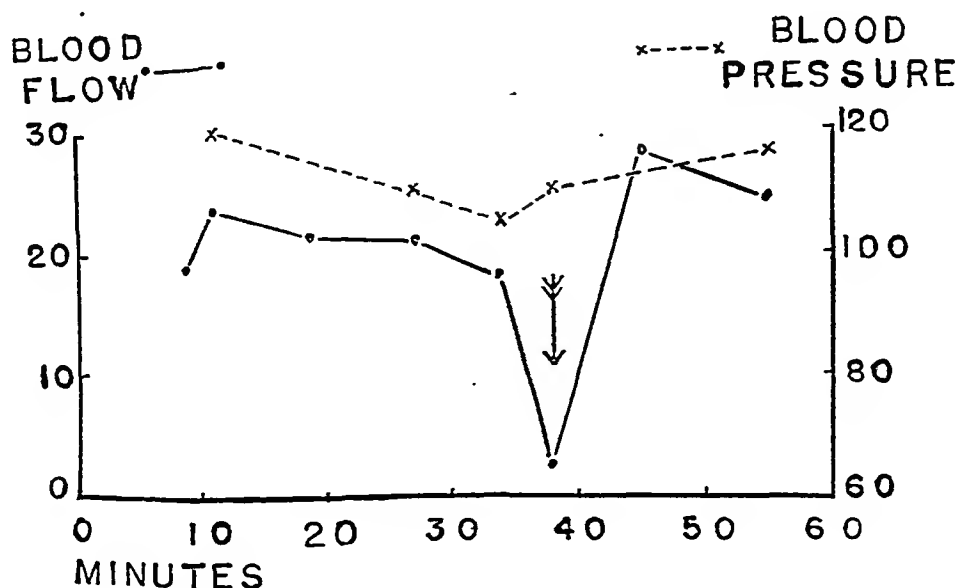


FIG. 7. EFFECT OF DILATING PYLORIC SPHINCTER UNDER AVERTIN AND ETHER ANESTHESIA ON THE VOLUME FLOW OF BLOOD THROUGH THE HAND MAINTAINED AT CONSTANT TEMPERATURE (34.7° C.).

Solid line: blood flow; interrupted line: blood pressure. Ordinates: cubic centimeters of blood flow per 100 cc. hand volume per minute and blood pressure, mm. mercury. Abscissae: time, minutes. The arrow indicates the time of dilatation of the pyloric sphincter.

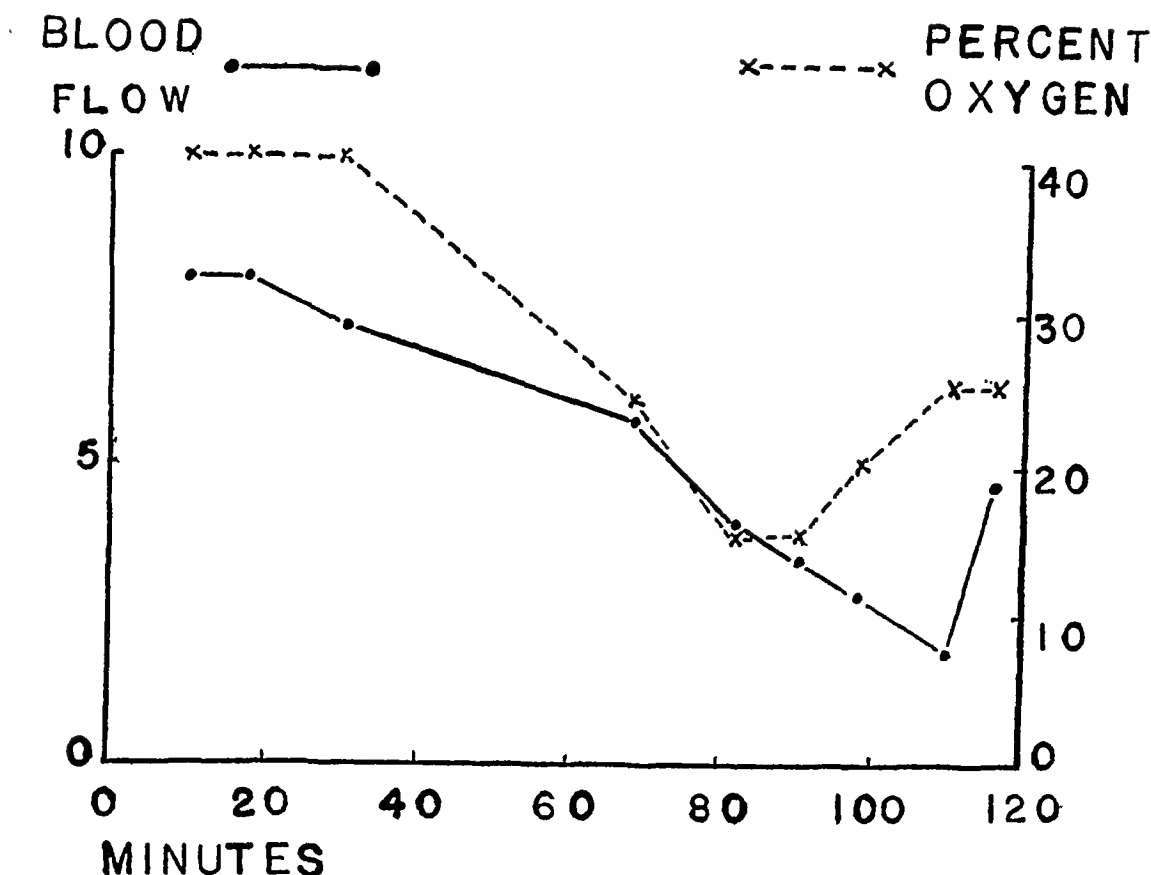


FIG. 8. EFFECT OF DECREASING THE CONCENTRATION OF OXYGEN IN AN OXYGEN TENT ON THE VOLUME FLOW OF BLOOD THROUGH THE HAND MAINTAINED AT CONSTANT TEMPERATURE ( $32.4 \pm .2^\circ \text{C.}$ ).

Solid line: blood flow; interrupted line: per cent oxygen in tent. Ordinates: cubic centimeters of blood flow per 100 cc. hand volume per minute, and per cent oxygen mixture in tent. Abscissae: time, minutes.

dration, was almost invariably present in the patients who were in poor condition. The effect of restitution of a diminished blood volume on the peripheral blood flow is pictured in Figure 9. This patient, M. O., was apparently suffering from intestinal obstruction. At the time she was first observed, she presented the classical picture of shock. Her temperature was  $101.6^\circ \text{F.}$ , her pulse 138, respiration 20 and blood pressure 78/60. Her extremities were cold. She was very restless and complained of severe thirst. At  $37^\circ \text{C.}$  the volume flow of blood through her hand was only 1.6 cc. per minute per 100 cc. hand volume. Reactive hyperemia raised the flow to 3.6 cc. per minute. Her venous blood was 41.2 per cent oxygenated. She was given 500 cc. of 6 per cent acacia in normal saline intravenously. As shown in Figure 9, her blood flow increased gradually to 7.6 cc. per minute. At the end of the injection her extremities were warm and dry.

Her blood pressure had risen to 140/70 and her pulse had fallen to 116. Now, after release of a tourniquet applied for 5 minutes, the circulation increased to 20 cc. per minute, in comparison with 3.6 cc. per minute 2 hours earlier. She relapsed into shock again a few hours later. Post-mortem examination revealed a volvulus of the entire small intestine. There were 2 liters of fluid present in the peritoneal cavity. The protein concentration of this fluid was 3.8 per cent. Her blood volume had probably been reduced by this loss of blood plasma into the peritoneal cavity.

#### DISCUSSION

It has previously been shown (2) that the circulation to the hand is under a dual control. Through the vasomotor nerves, the flow of blood is modified in accordance with the needs of the body as a whole. After removal of this control

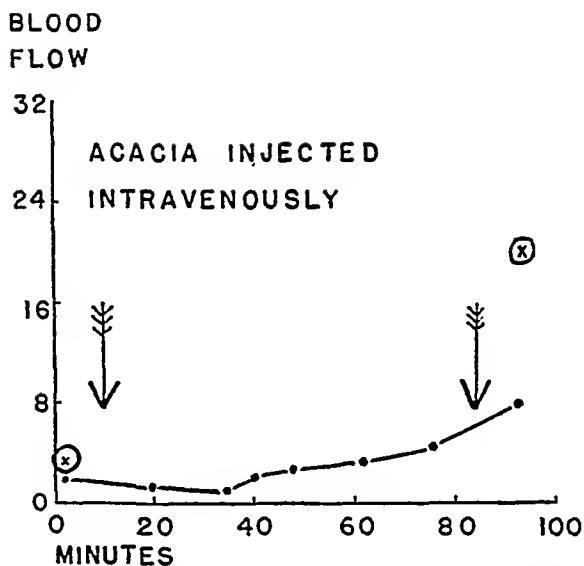


FIG. 9. EFFECT OF AN INTRAVENOUS INJECTION OF 500 CC. OF 6 PER CENT ACACIA IN NORMAL SALINE ON THE VOLUME FLOW OF BLOOD THROUGH THE HAND MAINTAINED AT CONSTANT TEMPERATURE ( $30.6 \pm .2^{\circ} \text{C.}$ ).

Ordinates: cubic centimeters of blood flow per 100 cc. hand volume per minute. Abscissae: time, minutes. The crosses surrounded by the circles indicate the extent of the reactive hyperemia before and after the injection. Between the arrows the patient received intravenously 500 cc. of 6 per cent gum acacia in normal saline solution.

by sympathectomy, the second factor is disclosed. The blood flow is then determined by the metabolic demand of the tissues. When the metabolism of the tissues is increased, by raising the temperature of the hand, the blood flows more rapidly. After release of a tourniquet, the blood flow is increased until the circulatory debt has been repaid.

Through vasoconstriction, the body is enabled to mobilize its circulation for the conservation of heat (8) or to divert the blood to the more vital centers in an emergency (9). At the same time, the circulation to the peripheral regions may be seriously reduced.

In the present experiments, the metabolic demands of the tissues of the hand were stabilized by maintaining it at constant temperature. It was then shown that traumatic stimuli, such as cold (see Figure 1), pain (see Figure 2) and fear (see Figure 3), produced a decrease in the volume flow of blood. These quantitative findings confirm the qualitative observations of many investi-

gators. The blood flow was studied with the metabolic needs stabilized in order to illustrate to what extent the demands of the peripheral tissues might be sacrificed to the needs of the body as a whole.

With asphyxia, a variable effect was observed (see Figure 4, and Table I). In the hand, with vasomotor control intact, a decrease in blood flow was observed in 6 cases, while an increase in circulation was found in 11 cases. Adequate confirmation for either result is furnished by the literature on the subject. Some investigators (10) have found an increase, while others (11) have noted a decrease in the peripheral circulation. The explanation for these conflicting observations, we believe, is afforded by the experiment illustrated in Figure 4. The blood flow decreased in regions subject to vasomotor control. It was simultaneously increased in the sympathectomized hand. It will be observed in Table I that the systolic blood pressure was raised but slightly, while the diastolic blood pressure more frequently was lowered during the period of asphyxia. In the 12 experiments in which the blood pressure was determined, the maximum rise in systolic pressure was only 10 mm. of mercury. In Experiment 16, while the blood flow increased by 50 per cent as a result of the asphyxia, the blood pressure actually declined. It is unlikely that the head of arterial pressure affected the results. It is recognized that asphyxia stimulates the sympatho-adrenal system (4). The resultant vasoconstriction reduces the circulation. Asphyxia of the degree employed in these experiments, however, also decreased the oxygen tension in the arterial blood (12, and cf. Experiments 4, 9 and 15, Table I). According to the concept previously established, (2), the flow of blood is modified by the metabolic demand of the tissues. With less oxygen available per unit volume of blood during asphyxia, an additional supply of blood would be required in order to maintain an equilibrium in the tissues. Two forces are therefore simultaneously called into play—reflex vasoconstriction and local vasodilatation. The final result, so far as the circulation through the hand is concerned, depends upon which of the two forces is dominant. In these experiments no clear indication was found as to which reaction would prevail, although when the subject was

apprehensive, a decreased blood flow was generally observed (cf. Figure 3). As far as the harmful effects of asphyxia on the oxygen supply to the tissues is concerned, the results are clear. Not only does the reduced oxygen saturation of the arterial blood necessitate a more rapid circulation, but through reflex vasoconstriction the supply of blood at the same time may even be reduced.

A reduction in the circulating blood volume has been recognized as a dominant feature in surgical shock since the work of Keith (13) and Robertson and Bock (14). Because the factors recognized to be of significance in the production of shock, such as cold, pain, fear, asphyxia, hemorrhage and dehydration are also stimulants of the sympathetic nervous system, the hypothesis was advanced (15) that the reduction in blood volume found in shock, where it could not be accounted for by hemorrhage or transudation (16), might be the result of sympathetic hyperactivity. In a series of experiments on cats, a decrease in blood volume was found to result from prolonged hyperactivity of the sympathetic nervous system. Since a reduction of the circulation leads to tissue anoxemia and an increase in the permeability of the capillaries (17), it was suggested that vasoconstriction with the attendant reduction of blood flow to large areas of the body might be the mechanism for this decrease in blood volume. The experiments here reported on the effect of cold, pain, fear, and asphyxia on the volume flow of blood through the hand indicate to what extent vasoconstriction may deprive the tissues of an adequate circulation.

The decreased volume of blood flow to the hand found in cases of surgical shock (Figure 6) is an objective record of a well recognized clinical observation. In experimental shock, produced by a variety of methods, Erlanger and his collaborators (1) invariably found an increased peripheral resistance. A diminished rate of blood flow through the salivary gland from hemorrhage and tissue abuse was consistently observed by Gesell (18). These investigators concluded that a reduced circulation is of fundamental importance in the development of shock.

If the volume flow of blood is decreased while the oxygen consumption of the tissues remains the same, it is to be expected that more oxygen

should be removed from the blood during its passage through the tissues. Consequently, the oxygen content of the venous blood should be decreased. The observations reported above in Figure 6 and Table II accord with this expectation. In the more severe cases of shock, the oxygen saturation of the venous blood was below 50 per cent. These findings are in accord with the experimental observations of Aub and Cunningham (19) and Blalock and Bradburn (20).

The inability of the tissues to obtain an adequate supply of blood was indicated by the observations on reactive hyperemia. In the normal subject (Figure 5), the circulation increased rapidly after release of the tourniquet. In patients who were in shock, a comparable increase was not observed (Figure 6 and Cases 4, 5, 7, 8, and 9, Table II). In spite of the fact that the needs of the tissues of the hand were increased during the time that the tourniquet was applied, reflex vasoconstriction was sufficiently powerful to prevent an effective increase in blood flow after the arterial circulation had been released. Patient J. M. (Case 8, Table II) furnishes an illustration of this phenomenon. Before operation, although he was in poor condition, his blood flow increased to 21 cc. per minute during the reactive hyperemia which resulted from application of the tourniquet for 5 minutes. The following day, at the end of the exploratory laparotomy, the maximum increase in blood flow was only to 2.2 cc. Four hours later, the reactive hyperemia had increased to 9.0 cc. and in 3 days to 17 cc.

This patient also illustrates the fact that the arterial blood pressure does not necessarily indicate the adequacy of blood flow to the tissues. Before operation his blood pressure was 100/62 and his blood flow 8 cc. per minute. At the end of operation even though his blood pressure had risen to 120/90 his blood flow had decreased to 1.3 cc. per minute. Clinically he presented the picture of surgical shock. His pulse was feeble, his color grey, and his skin cold and moist. Through vasoconstriction during the operation his blood pressure had been maintained but at the expense of his peripheral tissues. The blood pressure reading presented a false indication of his general condition. Four hours later, although his blood pressure had fallen to 86/56, his blood

flow had increased to 4 cc. per minute. In 3 days it had risen to the preoperative level, 8 cc. per minute, and he progressed to final recovery. The blood pressure in Patient A. B. (Case 11, Table II) was only 80/40, yet the circulation at 13 cc. per minute was quite adequate. In comparison, Patient M. J. D. (Case 6, Table II), at the conclusion of operation had a blood pressure of 125/80, but her blood flow was only 1.6 cc. per minute. She went on to death in 10 hours. The blood pressure is an expression of the quantity of blood in the circulation and of the peripheral resistance. If the blood volume is reduced, the blood pressure may still be sustained by increasing the peripheral resistance through vasoconstriction. With vasoconstriction, however, the nutrient flow to the tissues becomes reduced. If attention is focused chiefly on the level of the arterial pressure, the blood supply to the tissues, the ultimate goal of the circulation, may be overlooked.

The use of adrenalin or ephedrin in the treatment of surgical shock furnishes an example of this fact. The blood pressure may be increased, but only at the expense of the blood flow. The observations that adrenalin can produce shock (21) and cause a reduction in blood volume (15) accord with the clinical opinion as to its therapeutic ineffectiveness. Where the lowered blood pressure is the result of a reduced volume of blood in the vascular bed, physiological treatment necessitates the administration of blood or some blood substitute. The results of an intravenous injection of acacia in the treatment of a reduced plasma volume is illustrated in Figure 9. A comparable reaction from the administration of blood or acacia was noted by Gesell (18) in experimental shock.

The objection may be raised that these studies on the blood flow through the hand need not necessarily apply to the circulation through other areas of the body, such as the splanchnic area and the muscles. It would be difficult, if not impossible, in man, to study the flow of blood in these areas. We have, however, the clinical evidence of constricted vessels in the splanchnic area and the periphery (22). Finally, we have the low oxygen content of the mixed venous blood (20) and the decreased cardiac output (23) in experimental shock. These observations indicate that

the reduced blood flow in the hand, observed in clinical cases of surgical shock, represents a deficiency of circulation which is probably general throughout the body.

The "emergency function" of the sympatho-adrenal system was first emphasized by Cannon in 1914 (24). According to this concept, the sympathetic nervous system is thrown into activity when the organism is exposed to danger or to the expectation of harm. Under such circumstances, it enables the organism to survive. Experiments on cats which have survived total removal of the sympathetic nervous system have demonstrated that they can no longer adjust to various stresses in a normal manner. They readily succumb to cold and to excessive heat (25). Struggle causes them to faint (26). They are extremely sensitive to hemorrhage (27) and to asphyxia (28). The sympathetic nervous system appears to be necessary to enable the organism to adjust itself to an emergency. In a crisis, the non-essentials are sacrificed. The experiments and observations which have been presented in this paper indicate in what manner the needs of the tissues, not essential for the immediate survival of the body as a whole, may be sacrificed in an emergency. But if the reactions to the crisis are too severe and protracted, then vasoconstriction, the very mechanism by which the organism strives to survive, may lead to its destruction.

#### SUMMARY

1. Traumatic stimuli, such as cold, fear, and pain reduced the volume flow of blood through the hand maintained at constant temperature. The decrease in blood flow which resulted from intestinal manipulation was not prevented by general anesthesia.
2. Asphyxia brought about an increase in blood flow in the sympathectomized hand in 5 cases. In the normal hand, the blood flow was increased in 11 cases and decreased in 6 cases. The mechanism for these reactions is discussed.
3. The blood flow through the hand in clinical cases of surgical shock was markedly reduced.
4. The low oxygen saturation of the venous blood indicated the severity of the tissue asphyxia.
5. The reactive hyperemia which followed occlusion of the circulation for 5 minutes in cases of shock was slight and of short duration.

## CONCLUSIONS

Surgical shock is the clinical manifestation of a process which has its origin in the physiological reactions of the body to various traumatic stimuli. These reactions preserve the organism through diversion of the blood supply to the vital centers. As a consequence, the outlying tissues become deprived of adequate nutrition. Recovery from the process of shock is associated with progressive improvement in the circulation of blood to the periphery. The therapy of surgical shock should be directed toward the reestablishment of an adequate supply of oxygenated blood to the body tissues.

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# THE GLYCEMIC RESPONSE TO ISOGLUCOGENIC QUANTITIES OF PROTEIN AND CARBOHYDRATE

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In past years several objections to the use of a diet high in protein for the diabetic patient have been forwarded. These have fallen into three main groups—first, that in some way protein exerted a specific action, the result of which was to interfere with the mechanism of sugar utilization (1, 2, 3, 4); secondly, that the specific dynamic effect of protein in increasing heat production was wasteful in terms of total energy expenditure and as such should be minimized (1, 4, 5, 6); and, thirdly, that protein constituted a large source of endogenous glucose and therefore should be carefully curtailed in the diabetic diet. The latter is the only one that has in any measure stood the test of time as far as practical diabetic management is concerned (7, 8, 9). It is our purpose to show that it is an advantage to the diabetic to derive a large part of his total metabolic glucose from protein foods.

It is a fact that during the metabolism of protein there occurs a yield of glucose which approximates 50 per cent of the weight of the ingested protein (10, 11, 12, 13, 14, 15). Janney (16) working with isolated proteins found that the glucose liberated varied from 48 per cent to 80 per cent. It is reasonable to state that 50 per cent represents a good average figure in calculating diets in which the total intake of protein is made up of a mixture of many single proteins.

Since protein in the diet represents a large source of glucose, it was decided to compare the blood sugar levels and glycosuria produced by ingestion of equivalent amounts of glucose derived on the one hand from protein and on the other from glucose and carbohydrate foods. The studies were made on fifteen diabetic patients and three normal fourth year medical students. Each subject was maintained aglycosuric for at least two weeks before the study was begun.

## METHOD

The blood sugar level in the postabsorptive state was determined. At this time glucose was absent from all of the urine specimens. A breakfast consisting of 2 grams of protein per kilogram of body weight was given. The source of protein was lean beef from which all visible fat had been removed. This was ground and fried as hamburger steaks, using a minimum of butter in this process. The beef under these conditions contained 5 to 6 per cent of fat and 20 to 22 per cent of protein.

The time taken for ingestion of the meat varied from seven to twenty minutes. Blood and urine specimens were collected hourly for eight hours. Timing was begun immediately after the conclusion of breakfast.

Blood sugar was determined by the Benedict (17) method and blood urea nitrogen by the Van Slyke (18) urease method. Urinary sugar was determined by the Benedict (19) method and urinary nitrogen by the Kjeldahl method.

On another day a similar procedure was followed, but this time the breakfast consisted of one gram of carbohydrate per kilogram of body weight. This meal was given as glucose or as carbohydrate food calculated to yield this amount of glucose. When carbohydrate was used, specimens were collected for from three to four hours. In several subjects the response to various carbohydrate foods yielding equivalent amounts of glucose was compared with that obtained when the glucose was derived from protein. It is assumed that protein yields, during metabolism, glucose equal to 50 per cent of its weight.

## RESULTS

Figures 1 and 2 represent the results obtained from the normal group, showing that the response to ingestion of glucose is the expected one with



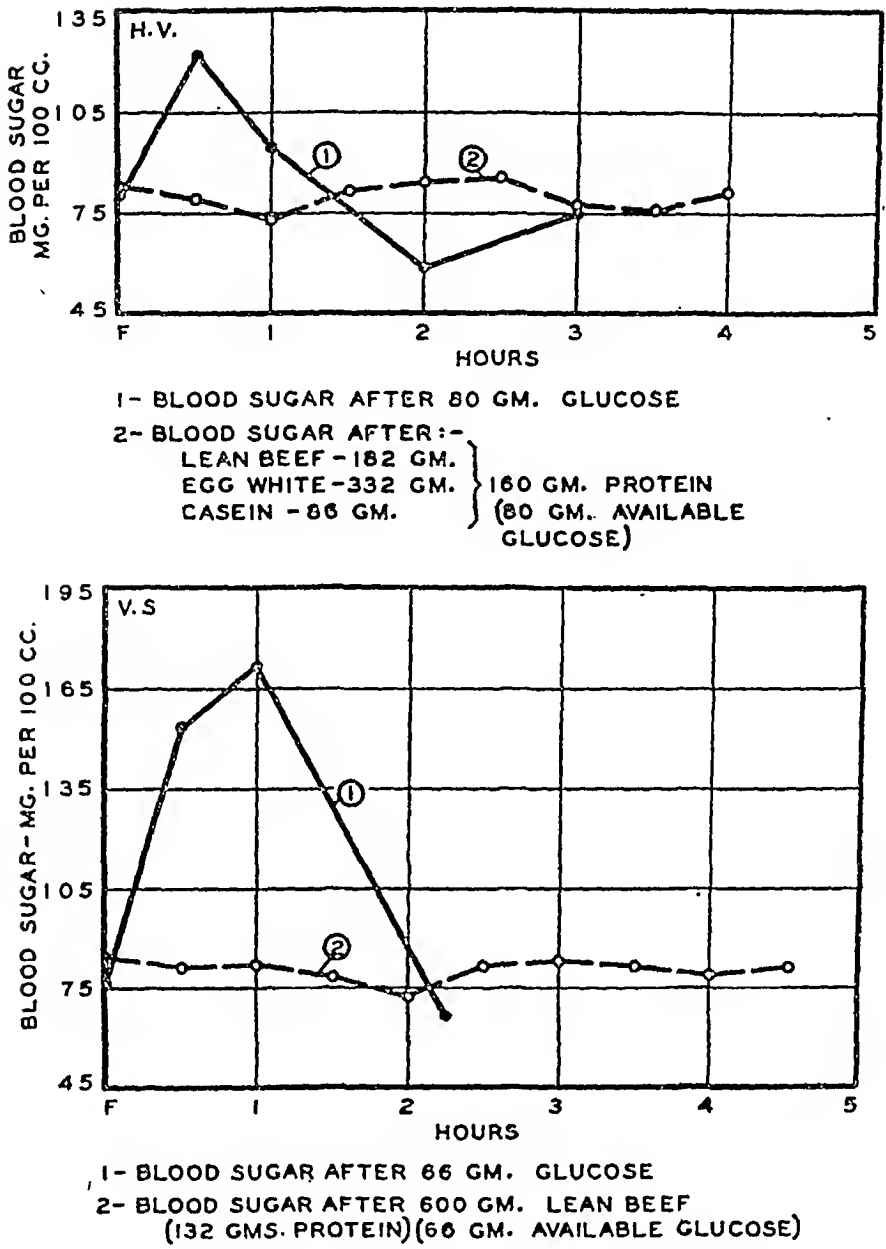


FIG. 1. BLOOD SUGAR CURVES IN NORMAL SUBJECTS, V. S. AND H. V.

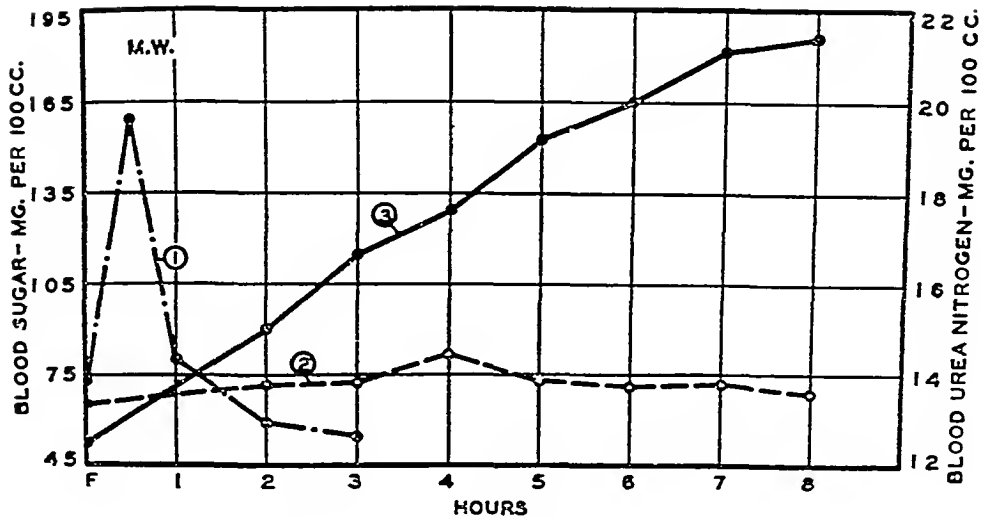
the usual variation. When an equivalent amount of glucose is derived from protein, however, the blood sugar curve remains flat. The rising blood urea nitrogen in Figure 2 indicates that protein is being metabolized. During the same period the blood sugar does not rise significantly.

Figures 3 and 4 show the results obtained in two diabetic subjects. A diabetic response to the ingestion of glucose is obtained. The curves after protein ingestion, however, remain essentially flat for an 8 hour period. Again, the rising blood urea nitrogen indicates protein utilization. The other curves show the values obtained after ingestion of equivalent amounts of glucose de-

rived from various carbohydrate foods. These curves resemble to a large degree those obtained after glucose ingestion.

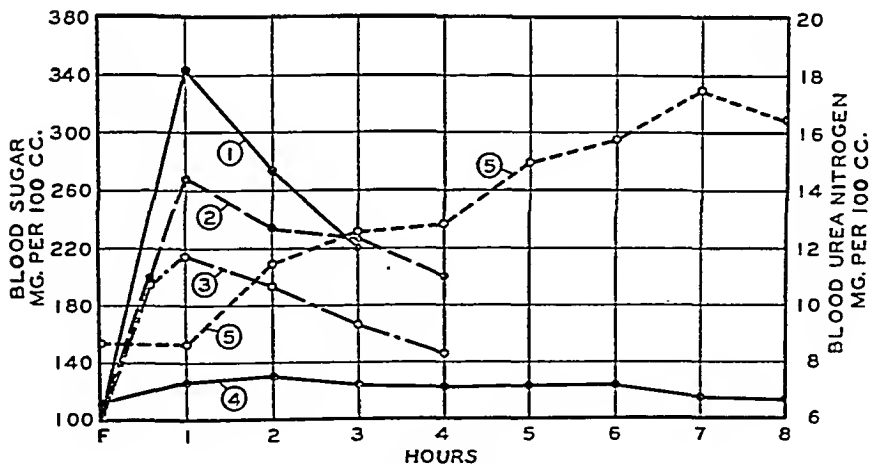
This confirms the recent observations of Wishnofsky and Kane (20) on the effect of equivalent amounts of dextrose and starch on glycemia and glycosuria. A marked glycosuria was found, in our studies, after ingestion of glucose or carbohydrate, while there was none after the protein meal.

Table I presents in quantitative terms the glycosuria found during the experimental period illustrated by Figure 4. When glucose or soluble starch was fed, approximately one-third of the



- 1- BLOOD SUGAR AFTER 66 GM. GLUCOSE  
 2- BLOOD SUGAR AFTER 600 GM. LEAN BEEF (132 GM. PROTEIN)  
 (66 GM. AVAILABLE GLUCOSE)  
 3- BLOOD URÉA NITROGEN AFTER 600 GM. LEAN BEEF  
 (132 GM. PROTEIN)

FIG. 2. BLOOD SUGAR AND BLOOD UREA NITROGEN IN NORMAL SUBJECT M. W.



- ①-BLOOD SUGAR AFTER 53 GMS. GLUCOSE  
 ②-BLOOD SUGAR AFTER 100 GMS. BREAD & 25 GMS. BUTTER  
 (53 GMS. AVAILABLE GLUCOSE)  
 ③-BLOOD SUGAR AFTER 370 GMS. APPLE (53 GMS. AVAILABLE GLUCOSE)  
 ④-BLOOD SUGAR AFTER 485 GMS. LEAN BEEF (106 GMS. PROTEIN)  
 (53 GMS. AVAILABLE GLUCOSE)  
 ⑤-BLOOD URÉA NITROGEN AFTER 485 GMS. LEAN BEEF (106 GMS. PROTEIN)

FIG. 3. BLOOD SUGAR AND BLOOD UREA NITROGEN IN CASE II

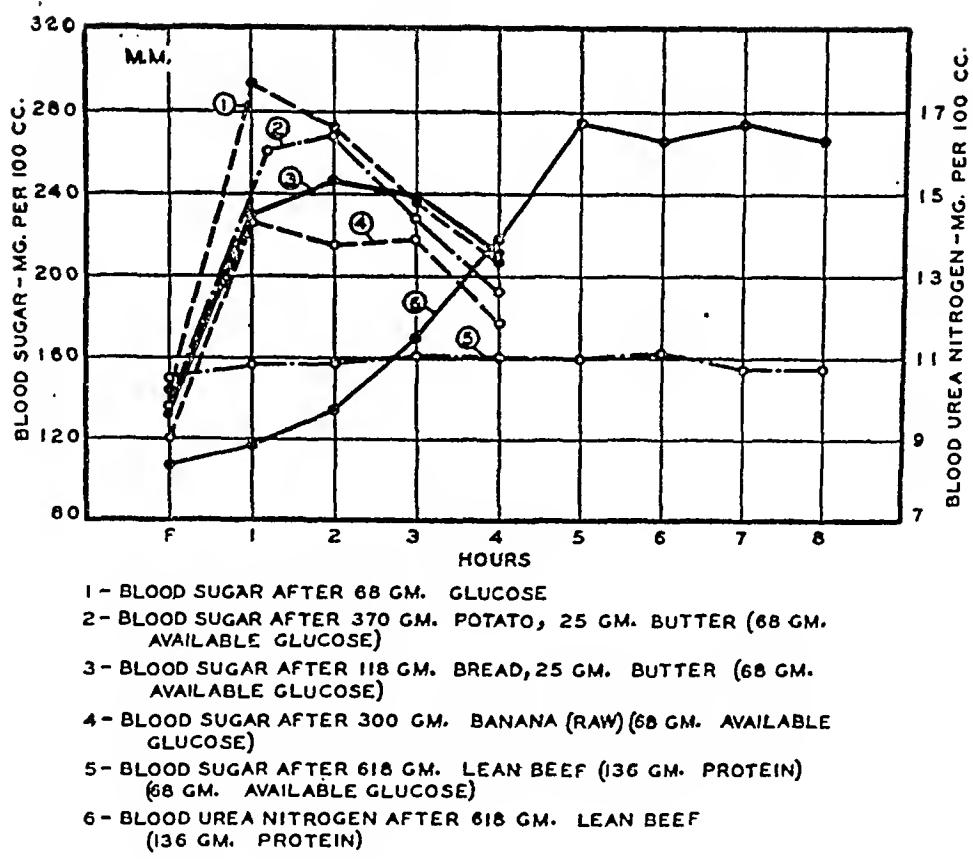


FIG. 4. BLOOD SUGAR AND BLOOD UREA NITROGEN IN CASE I

ingested glucose appeared in the urine in 4 hours. In the case of raw starch (raw fruits) a lesser amount of glycosuria appeared in the four hour period. During the entire 8 hour period following ingestion of 617 grams of lean beef, however, there was no glycosuria.

Detailed data of these and the other subjects studied are seen in Table II. The average maximal increase in blood sugar above the fasting level after ingestion of carbohydrate was 160

mgm. per 100 cc. of blood and was invariably attended by glycosuria. When an equivalent amount of glucose was derived from protein the average maximal increase was 37 mgm. per 100 cc., and little or no glycosuria resulted. It is noteworthy, too, that the small increase in blood sugar after protein feeding appears to parallel the severity of the disease much more closely than does the rise after the ingestion of glucose.

DISCUSSION

These studies demonstrate that ingested protein causes a very much smaller increase in the blood sugar than results from an equivalent amount of glucose or carbohydrate food. We believe that the explanation involves a principle which has received very little attention.

We are accustomed to measure the capacity of a diabetic to dispose of glucose in terms of the total number of grams of glucose released from his diet in 24 hours. The number of grams of glucose that just fails to produce glycosuria is taken to be a measure of the largest amount of glucose that the patient can utilize in a 24 hour period. This conception takes no account of the

TABLE I  
*Glycosuria of various foods in diet of Case I (M. M.)*

Food ingested	Amount	Available glucose	Urinary glucose (first 4 hours)	
	grams	grams	grams	per cent of ingested glucose
Lean beef	617	68	0*	0.*
Orange juice	200	68	23.0	33.8
Dextrose	47			
Potato (steamed)	370	68	22.5	33.0
Butter	25			
Bread	118	68	23.3	34.2
Butter	25			
Banana, raw	300	68	11.2	16.4

\* No urinary glucose during entire 8 hour experimental period.

rate at which the total amount of glucose produced in 24 hours enters the blood stream. Since maximal tolerance without glycosuria depends upon the *rate* at which the body is able to remove glucose from the blood stream by oxidation, deposition as glycogen, and transformation to fat, the time element assumes an importance at least equal to that of total yield of glucose in grams.

Doyon and Dufourt (21) in 1901 first noted that the rate of injection of glucose into the blood stream was a very important factor in the production of glycosuria. They found that a much

greater quantity of glucose could be given per unit of time without causing glycosuria if the rate of injection was slow. Blumenthal (22) in 1905 did an interesting experiment bearing on the influence of the time element upon the production of glycosuria. He ascertained the dose of glucose that could be given intravenously every fifteen minutes without producing glycosuria. A dose slightly in excess of this always produced glycosuria. After having found the maximal dose that failed to produce glycosuria when given every fifteen minutes, he then gave one of

TABLE II  
*Data from all of the subjects studied*

Case number	Weight	Age	Tolerance* (available glucose)	Food ingested				Blood sugar		Glycosuria †		Maximal increase in blood urea nitrogen	Urinary nitrogen (8 hour period)	
				Type	Amount	Protein	Available glucose	Fasting	Maximal increase				Fast-ing	During test
	kgm.	years	grams		grams	grams	grams	mgm. per 100 cc.	mgm. per 100 cc.		grams	mgm. per 100 cc.	grams	grams
DIABETICS														
I M.M.	68	21	110	Lean beef	618	136	68	149	11	0		8.4	0.8	5.7
				Dextrose	68		68	144	148	++++	23.0			
				Bread	118		68	132	114	++++	23.3			
				Butter	25		68	135	133	++++	22.5			
				Potato	370		68	120	106	++++	11.2			
				Butter	25		68							
				Banana	300		68							
II	53	23	125	Lean beef	485	106	53	112	16	0		8.8	1.7	4.4
				Dextrose	53		53	105	240	++++	7.8			
				Bread	100		53	108	159	++++				
				Butter	25		53	109	104	++				
				Apple (raw)	370		53							
III	46	16	50	Lean beef	418	92	46	87	33	0		7.0	1.4	7.5
				Lean beef	418	92	46	100	51	0		10.7	2.9	8.7
				Dextrose	46		46	168	156	++++				
IV	60	52	95	Lean beef	430	94	47	89	43	0		6.4		
				Dextrose	47		47	104	132	++++	12.2			
V	51	47	65	Lean beef	480	102	51	111	46	0		8.3		
				Dextrose	51		51	132	158	++++				
VI	65	20	50	Lean beef	590	130	65	65	57	0				
				Dextrose	65		65	60	192	+++				
VII	38.2	14	80	Lean beef	347	76.4	38.2	136	26	0		10.5	2.2	6.5
				Dextrose	38.2		38.2	106	142	+++	4.8			
VIII	69	46	300	Lean beef	627	138	69	98	6	0		9.8	1.7	5.2
				Dextrose	69		69	104	84	+++				
				Bread	135		69	102	41	0				
				Butter	25		69							
IX	55	17	80	Lean beef	500	110	55	110	69	+	0.3	5.9	3.1	11.8
				Dextrose	55		55	79	151	++++	9.1			
				Bread	118		55	94	169	++++	5.4			
				Butter	25		55							

\* Tolerance here is meant to represent the maximal number of grams of available glucose that the diabetic can utilize without glycosuria in a 24 hour period without the aid of exogenous insulin when he is given the usual three diabetic meals a day. (The usual diabetic diet contains about two-thirds of a gram of protein per kilo per day). This implies the absence of infection or other complication known to lower tolerance.

† During the protein test the presence or absence of glycosuria was followed for the entire 8 hour experimental period. After glucose or carbohydrate foods this was followed for only four hours and many subjects continued to have marked glycosuria after four hours.

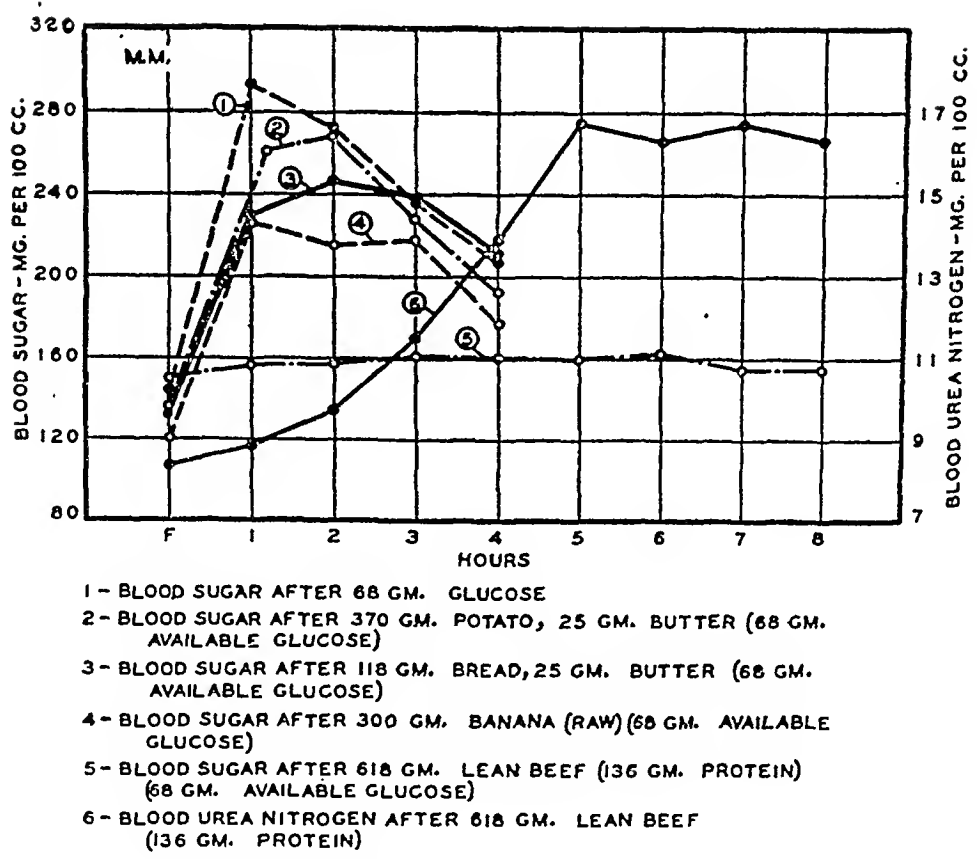


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				Dextrose	65		65	60	192	+++				
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				Bread	118		55	94	169	++++	5.4			
				Butter	25									

\* Tolerance here is meant to represent the maximal number of grams of available glucose that the diabetic can utilize without glycosuria in a 24 hour period without the aid of exogenous insulin when he is given the usual three diabetic meals a day. (The usual diabetic diet contains about two-thirds of a gram of protein per kilo per day). This implies the absence of infection or other complication known to lower tolerance.

† During the protein test the presence or absence of glycosuria was followed for the entire 8 hour experimental period. After glucose or carbohydrate foods this was followed for only four hours and many subjects continued to have marked glycosuria after four hours.

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TABLE II—Continued

JEROME W. CONN AND L. H. NEWBURGH  
 TABLE II—Continued

Case number	Weight	Age	Tolerance* (available glucose)	Food ingested			Blood sugar		Glycosuria †		Maxi- mal in- crease in blood urea nitro- gen	Urinary nitrogen (8 hour period)	
				Type	Amount	Pro- tein	Avail- able glucose	Fasting				Fast- ing	During test
X	kgm. 72	years 47	grams 80	Lean beef Dextrose Bread Butter	grams 654 72 124 125	grams 144	grams 72 72 72	mgm. per 100 cc. 90 89 66	mgm. per 100 cc. 64 208 211		mgm. per 100 cc. 9.3	grams 2.7	grams 11.4
XI	66	32	80	Lean beef Dextrose	610 66	132	72 72	90 89 66	64 208 211	++ ++++ ++++	4.1 20.0 10.8		
XII	72	29	0 (Total diabetic)	Lean beef Dextrose	615 67	135	72 72	90 89 66	64 208 211	++ ++++ ++++	4.1 20.0 10.8		
XIII	62	49	?	Lean beef Dextrose	560 62	124	66 66	101 115	75 163	++ ++++ ++++	7.5		
XIV	28	10	65	Lean beef Dextrose	254	56 not done	67.5 67	125 166	170 174	++ ++++ ++++	11.3	3.4	7.
XV	56	16	?	Lean beef Dextrose	509 56	112	62 62	128 142	34 138	0 ++++ ++++	9.4	3.2	5.9
							128 81	56	9 141	Tr. ++++ ++++	0.4 9.3 4.8	2.5 4.1	5.8 8.6
NORMALS													
H.V.	73	23	Normal	Lean beef Egg white Casein Dextrose	182 332 86 80	160	80 80	83 80	2 42	0 0			
V.S.	75	24	Normal	Lean beef Dextrose	600 66	132	66 66	84 76	-1 97	0 +			
M.W.	71	24	Normal	Lean beef Dextrose	600 66	132	66 66	66 74	16 82	0 +	8.9		

the series of injections 10 minutes after the pre-  
 ceding one. This provoked glycosuria. The next  
 injection at the regular time in the series  
 produce glycosuria.

Gray (24)

the series of injections 10 minutes after the preceding one. This provoked glycosuria. The next injection at the regular time in the series failed to produce glycosuria. The crest of the glycaemic wave produced by the irregular injection was higher than those which followed the regularly timed injections (every 15 minutes). The renal threshold was thus exceeded even though the total amount of glucose for the whole period was the same. Woodyatt et al. (23) in 1915 made the incisive statement, "Tolerance must be regarded as a velocity, not as a weight. It must be measured and expressed in grams of glucose per kilogram of body weight per hour of time or in other convenient units of weight and time."

It is not surprising, then, that the diabetic, whose capacity to utilize glucose is already damaged, when given his total 24 hour quantity of glucose in the form of three meals over a 10 hour period, has excessive hyperglycemia and glycosuria. Yet his true capacity for disposing of glucose in 24 hours may not have been exceeded. In 1922

Gray (24) reported better results in diabetics when the total quantity of food was divided into six meals than when three meals were given. This could easily be explained on the time factor alone.

In the process of protein metabolism, the complex protein molecule is split in the intestinal tract to amino-acids. These are absorbed into the blood stream and transported to the liver where oxidative deamination occurs. Here the glyco- genic amino-acids are split to form urea and glucose. That this process is a slow one is shown in the charts by the slowly rising blood urea nitrogen. Glucose is, therefore, liberated into the blood stream in this process at a slow and even rate over a prolonged period of time. Under these conditions the diabetic is able to utilize a greater total amount of glucose without glycosuria in the eight hour period. Therefore, the inability of a diabetic to dispose of large quantities of glucose is partially compensated if the glucose is presented for utilization slowly and evenly. There appears,

then, to be some advantage to the diabetic of this slow liberation of glucose from protein foods.<sup>1</sup>

### CONCLUSIONS

(1) A comparison was made between the glyceemic and glycosuric responses after ingestion of equivalent amounts of glucose derived from glucose per se, protein and carbohydrate foods in normals and in diabetics.

(2) Within the limits of these studies there is a decided advantage to the diabetic of deriving glucose from protein.

(3) The slow rate of liberation of glucose into the blood stream during protein metabolism is the explanation of the results obtained.

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<sup>1</sup> Studies are now being conducted with prolonged feeding of high protein diets to diabetics to determine the therapeutic significance of these observations.





# THE ADVANTAGE OF A HIGH PROTEIN DIET IN THE TREATMENT OF SPONTANEOUS HYPOGLYCEMIA

## PRELIMINARY REPORT

By JEROME W. CONN

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Several clinical procedures for the control of paroxysmal spontaneous hypoglycemia have been advocated, but therapeutic results, for the most part, have been disappointing. It is our purpose to review briefly the present status of medical treatment and to present evidence in support of a dietary régime which is successful and appears to be justified from a theoretical point of view.

Since Harris (1) first described the clinical syndrome of hyperinsulinism in 1924 the condition has been recognized with increasing frequency. Many reviews (2, 3, 4, 5, 6) comprising large numbers of cases have appeared in the more recent literature, due consideration having been given to both the medical and surgical aspects of the disease. Inasmuch as this report is concerned mainly with the treatment of the condition, a brief description of the clinical syndrome will suffice.

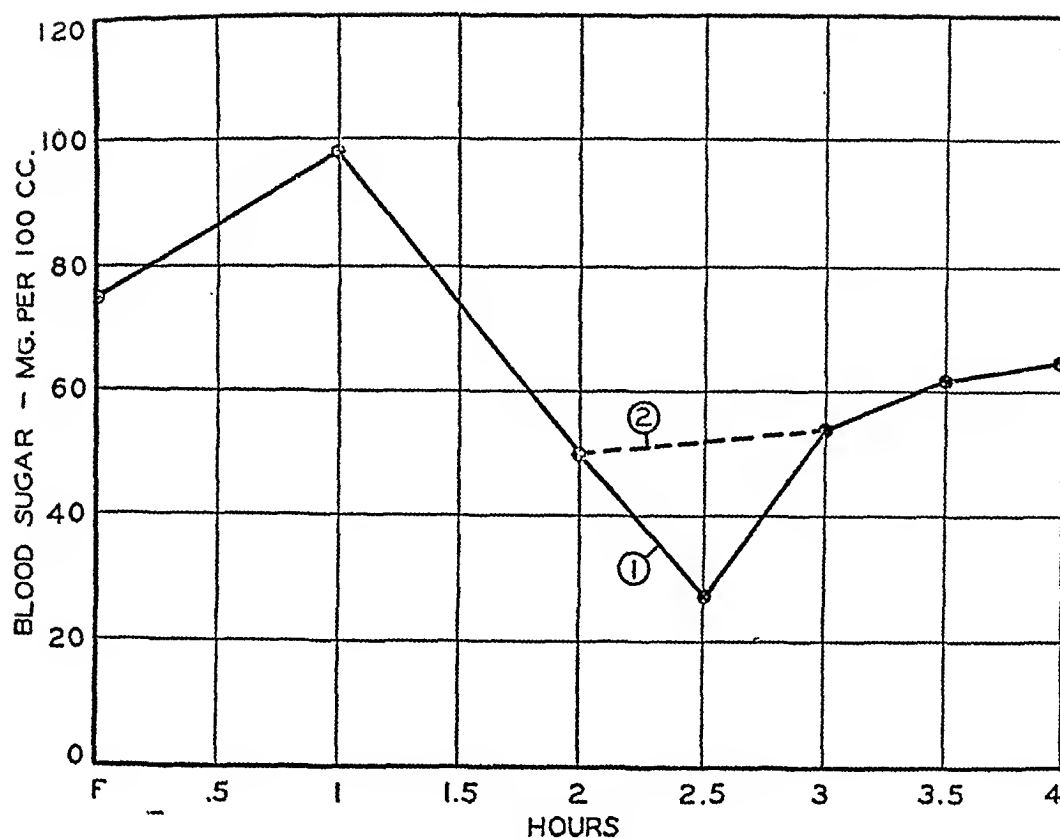
The disorder is characterized by the periodic occurrence of symptoms varying from weakness, hunger, trembling, excessive sweating, visual disturbances or giddiness, to temporary lapses in memory, changes in behavior and personality, convulsions and unconsciousness. These symptoms, which have come to be associated with hypoglycemia, are similar to those seen following the administration of an overdose of insulin. The symptoms usually occur three to four hours after meals or in the early hours of the morning. Patients often discover that the ingestion of food, particularly carbohydrate food, relieves the symptoms. The presence of an abnormally low blood sugar during an attack, and relief of the attack by ingested or intravenous glucose is sufficient to make the diagnosis. When, as is often the case, the physician fails to see the patient during an attack, the glucose tolerance test as a diagnostic aid is often helpful. Two to five hours after the ingestion of a standard amount of glucose (1.75 grams per kilo of body weight) these

patients exhibit a precipitous fall in the blood sugar to hypoglycemic levels. It must be remembered, however, that the hypoglycemia obtained in this way may be of short duration and may be missed if the usual hourly blood samples only are taken. This point is illustrated in Figure 1 which represents one of the curves obtained in Case I described below. We have, therefore, modified the test somewhat by taking blood samples every thirty minutes after the second hour for two to three hours.

A survey of the literature reveals that if the pancreases of these patients are examined, either surgically or at autopsy, about 50 per cent are found to have pancreatic abnormalities. (This statement excludes those hypoglycemic states which are due to other pathological processes, such as diseases of the liver (7, 8), pituitary (9), thyroid (10) or adrenal glands (11)). In the group which has abnormalities of the pancreas, islet cell carcinomas, adenomata of islet tissue and simply hypertrophy of the islets of Langerhans have been reported.

### *Surgical treatment*

When an unquestioned clinical diagnosis has been made there appears to be a 50 per cent chance that a pancreatic abnormality will be found at laparotomy. Exploratory laparotomy, therefore, seems to be indicated, especially if the patient is in the cancer age and if symptoms have been progressive and of relatively short duration. If a pancreatic tumor has been found and removed surgically, results have been excellent, with complete cure in many cases (12). If a grossly normal pancreas has been found, partial pancreatectomy has been advised (13) and done in many cases, with disappointing results as far as alleviation of hypoglycemic attacks is concerned.



1.- BLOOD SUGAR CURVE AS DETERMINED WITH  
30 MINUTE SAMPLES

2.- BLOOD SUGAR CURVE HAD HOURLY SAMPLES BEEN TAKEN

FIG. 1. GLUCOSE TOLERANCE TEST

### *Medical treatment*

One is left, then, with a group of patients who must be treated by medical measures because operation is not advised, is refused, or has been performed without alleviation of symptoms. Three types of medical management have thus far been advocated. The first and most obvious method of treatment was a high carbohydrate diet, suggested by the patients' own observations that food relieved symptoms. It was reasoned that if the subject were suffering from an excessive production of pancreatic insulin, a large amount of carbohydrate in the diet would have a tendency to neutralize this effect. Again results were disappointing. While some of the patients improved on this régime, many were unimproved or actually were made worse (14). It was soon realized that the ingestion of carbohydrate afforded an extra stimulus to pancreatic formation of insulin, which is already being produced in excessive amounts. Experimental evidence supporting this observation is afforded by Len-

nox (15). The pendulum then swung in the opposite direction and Waters (5) in 1931 advised strict curtailment of the carbohydrate in the diet, most of the calories being derived from fat. Following this a high fat, low carbohydrate diet with feedings divided into six daily meals was generally adopted. On this régime there was often prompt improvement; but while hypoglycemic attacks were diminished in number, they still occurred with alarming frequency.

The next and most recent therapeutic suggestion was that of John (16). He reasoned that if the sudden drop of the blood sugar to hypoglycemic levels was secondary to the pancreatic stimulation caused by the postprandial rise in blood sugar, then something which would prevent the hyperglycemia might also prevent the subsequent drop in blood sugar values. He, therefore, uses a low carbohydrate diet divided into three meals and gives about ten units of insulin one-half hour after each meal. He reports good results.

We (17) have shown that in both normals and diabetics the ingestion of large amounts of protein is followed by comparatively little or no rise in the blood sugar level. Yet protein during its metabolism yields approximately 50 per cent of its weight as glucose. When an equivalent amount of glucose is ingested as such or as carbohydrate food a significant hyperglycemia is produced.

Our interpretation of this difference in the glycemic response to isoglucogenic quantities of protein and carbohydrate is given in detail in the previous paper (17). Suffice it to say here that during the metabolism of protein, the large amount of glucose derived in this process is liberated into the blood stream at a slow and even rate over a prolonged period of time and fails to produce hyperglycemia.

It was realized, then, that the ingestion of large amounts of protein would supply glucose to the blood stream at a constant, slow rate, without the production of a hyperglycemia. This would be advantageous to the patient with hyperinsulinism in that it would not stimulate the insulogenic mechanism and yet would supply a source of glucose over a considerable period. The carbohydrate of the diet could be further restricted when necessary in severe cases. A large amount of glucose could be derived from protein. This glucose can be made available in sufficient amount to produce a fatty acid-glucose ratio that will prevent acidosis, even if no carbohydrate is included in the diet.

Three typical examples of spontaneous hypoglycemia were chosen for this study. All three came to the hospital complaining of attacks of convulsions and unconsciousness, symptoms which conform to the criteria, mentioned above, necessary for the diagnosis.<sup>1</sup> One of the three which will be discussed in detail is Weil's (14) case reported as "functional hyperinsulinism" in 1932.

*Case I (V. S.).* On admission to the hospital a provisional diagnosis of spontaneous hypoglycemia was made. The following is the response to the routine glucose tolerance test (1.75 grams glucose per kilogram of body weight given orally).

Fasting .....	74 mgm. sugar per 100 cc. blood
1 hour .....	115 mgm. sugar per 100 cc. blood
2 hours .....	70 mgm. sugar per 100 cc. blood
3 hours .....	40 mgm. sugar per 100 cc. blood

A repetition of this test gave the following results:

Fasting .....	75 mgm. sugar per 100 cc. blood
1 hour .....	98 mgm. sugar per 100 cc. blood
2 hours .....	50 mgm. sugar per 100 cc. blood
2½ hours .....	23 mgm. sugar per 100 cc. blood
3 hours .....	54 mgm. sugar per 100 cc. blood
3½ hours .....	62 mgm. sugar per 100 cc. blood
4 hours .....	65 mgm. sugar per 100 cc. blood

Figure 2 shows the comparative effects of ingestion of one gram of glucose per kilogram of body weight and 2 grams of protein per kilogram of body weight each yielding an equivalent amount of glucose in its metabolism. It is seen that after ingestion of glucose a hypoglycemic phase follows directly upon the hyperglycemic one. On the other hand, the curve following the ingestion of protein remains essentially flat over a period of eight hours. The gradual and prolonged rise of the blood urea nitrogen reflects the utilization of protein and the slow liberation of glucose into the blood stream.

*Case II (W. L.).* The history suggested spontaneous hypoglycemia. A routine glucose tolerance test gave the following response:

Fasting .....	82 mgm. sugar per 100 cc. blood
1 hour .....	208 mgm. sugar per 100 cc. blood
2 hours .....	120 mgm. sugar per 100 cc. blood
3 hours .....	30 mgm. sugar per 100 cc. blood

Figure 3 again demonstrates the absence of either hypoglycemia or hyperglycemia when the glucose is derived from protein. When, however, this same amount of glucose is given as such, both the hypoglycemic and hyperglycemic responses are obtained. The smaller amount of glucose ingested in this test probably accounts for the fact that the degree of pancreatic stimulation (15) and consequent hypoglycemia are less than those of the original test given above.

*Case III (L. J.).* This patient's early history with complete and thorough study is reported by Weil (14). This physician made the diagnosis of hyperinsulinism and prescribed treatment for this condition.

Briefly, a 32 year old, intelligent housewife, complained of faintness and weakness, occasionally accompanied by convulsions and unconscious spells, beginning in October, 1930. There have been 35 to 40 convulsive attacks since that time. Very low blood sugar values were found during attacks. She frequently returned to a normal state within a few minutes after glucose was given either intravenously or by mouth. Often when actual convulsions did not occur there were periods of weakness, trembling, sensorial clouding, loss of memory and giddiness. Although attacks usually occurred during sleep in the early hours of the morning, they sometimes came on during the day, three to four hours after the preceding meal. The attacks were frequently centered about the week of catamenia, although they had occurred at any time of the month.

<sup>1</sup> Since it is not the purpose of this report to enlarge on diagnostic criteria, details of the case histories are intentionally omitted to conserve space.

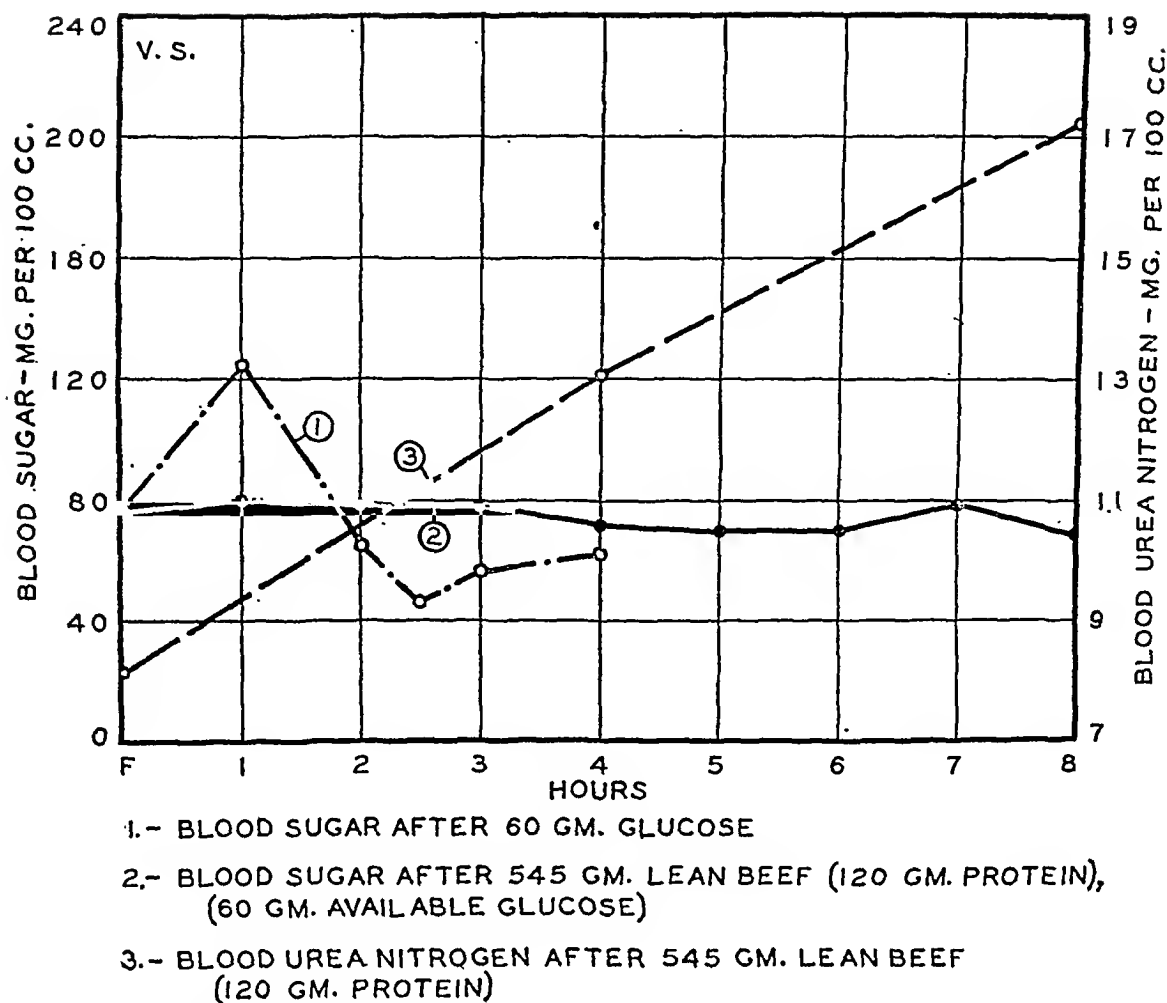


FIG. 2. BLOOD SUGAR AND BLOOD UREA NITROGEN CURVES IN CASE I

A relatively high carbohydrate diet had failed to effect improvement and had made the patient worse. A low carbohydrate, high fat diet (60 grams of protein, 250 grams of fat and 100 grams of carbohydrate) divided into 5 or 6 equal feedings prescribed by Weil improved the symptoms and reduced the frequency of attacks, but the patient was not relieved of these episodes.

She came to the University Hospital on October 18, 1935, stating that in spite of rigid adherence to her weighed diet her attacks were becoming more frequent and alarming. Between attacks she noticed marked faintness, restlessness and memory difficulty. Exploratory laparotomy was discussed with the patient, but not urged.

The blood sugar curves following ingestion of equivalent amounts of glucose as such and as derived from protein are shown in Figure 4. Following glucose ingestion a rise in the blood sugar is followed by a precipitous fall to 39 mgm. per 100 cc. After ingestion of protein, however, the blood sugar curve remained flat for an 8 hour period. The rise of the blood urea nitrogen again signifies protein utilization. It will be noted that there was very little drop in the blood sugar in the fasting state. It appears, then, that the hyperglycemia produced by the ingestion of glucose affords the stimulus for the secretion of insulin, which results in the secondary hypoglycemia.

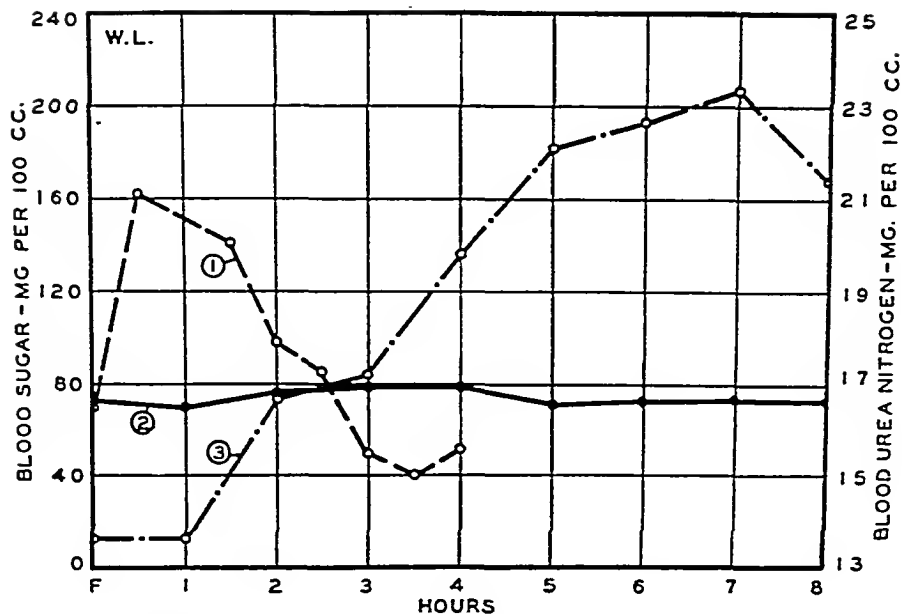
On February 7, 1936, the patient was instructed to use a diet containing 110 grams of protein, 150 grams of fat and 50 grams of carbohydrate, yielding a total of 2000 calories. It was felt from the experience with experimental periods, that the glucose would be delivered slowly over a sufficiently prolonged period to allow freedom from attacks even if only three meals a day were given. The diet, therefore, was divided into three equal meals to be taken at the usual times.

More than three months have elapsed. The patient has had no convulsions. She reports that she has felt much better in all respects. She no longer has the periods of faintness, weakness, restlessness and trembling complained of during the years of treatment with other dietary plans.

#### CONCLUSIONS

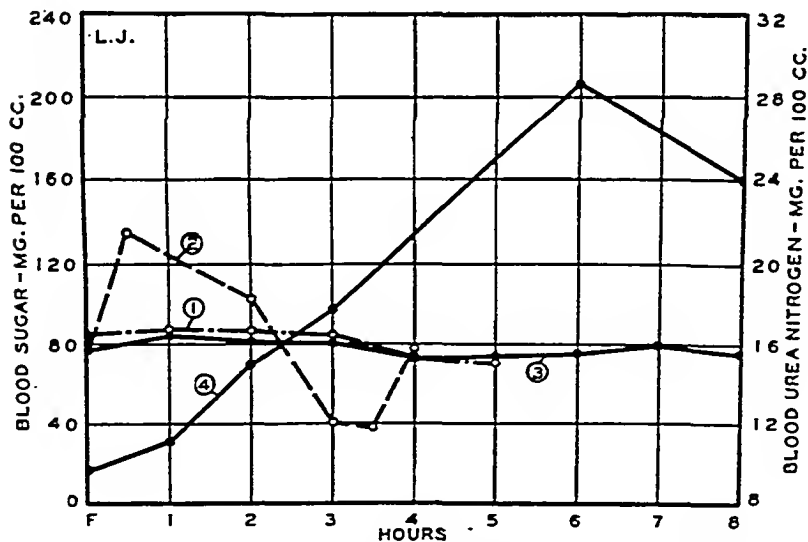
1. The slow rate at which glucose is liberated into the blood stream during the metabolism of protein is of advantage in the treatment of spontaneous hypoglycemia because—

(a) It causes no hyperglycemia and thus avoids excessive production of insulin and secondary hypoglycemia.



- 1.- BLOOD SUGAR AFTER 57 GM. GLUCOSE
- 2.- BLOOD SUGAR AFTER 518 GM. LEAN BEEF (114 GM. PROTEIN),  
(57 GM. AVAILABLE GLUCOSE)
- 3.- BLOOD UREA NITROGEN AFTER 518 GM. LEAN BEEF  
(114 GM. PROTEIN)

FIG. 3. BLOOD SUGAR AND BLOOD UREA NITROGEN CURVES IN CASE II



- 1.- BLOOD SUGAR FASTING (TEST BEGUN AFTER 12 HOUR FAST)
- 2.- BLOOD SUGAR AFTER 59 GM. GLUCOSE
- 3.- BLOOD SUGAR AFTER 545 GM. LEAN BEEF (120 GM. PROTEIN),  
(60 GM. AVAILABLE GLUCOSE)
- 4.- BLOOD UREA NITROGEN AFTER 545 GM. LEAN BEEF  
(120 GM. PROTEIN)

FIG. 4. BLOOD SUGAR AND BLOOD UREA NITROGEN CURVES IN CASE III

(b) It provides a source of glucose over a prolonged period of time.

(c) It allows in severe cases further restriction in carbohydrate than could otherwise be effected.

2. These facts justify the use of a diet high in protein and low in carbohydrate in the treatment of this condition.

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# THE METABOLISM OF HUMAN ERYTHROBLASTS

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The metabolism of human nucleated red blood cells has not previously been determined because of difficulties in procuring material. In most anemias the number of nucleated erythrocytes in the blood is so small in relation to the number of non-nucleated erythrocytes that the metabolism of the former is obscured by that of the latter. Moreover, it is impossible without damaging their very sensitive metabolic reactions to separate in sufficient amount the nucleated from the non-nucleated red blood cells and from the leukocytes.

The opportunity recently presented itself through the kindness of the Department of Pediatrics of examining at intervals of from three to five months the blood of a patient with erythroblastic anemia who had an extremely high proportion of nucleated red blood cells, 130,000 to 364,000 erythroblasts to 1.8 to 3.5 million non-nucleated erythrocytes in 1 c.mm. of blood. The patient was an eight year old boy of Greek parentage. One sister died at 2 years with symptoms similar to the patient's. Parents and a 3 year old brother are in good health. Since 1932 the patient has been given blood transfusions for his anemia regularly every few months. Splenectomy in 1933 was not followed by any evident improvement. At the time this study was begun there were 245,000 erythroblasts and 3.25 million non-nucleated red blood cells in 1 c.mm., that is, a percentage of 7 per cent of erythroblasts in his total red cell count. The percentage of reticulocytes was below 1 per cent. The leukocytes varied between 5,000 and 7,000 per c.mm.

## METHOD

To determine the exact metabolism of animal cells in vitro it is imperative to avoid injury and changes in the physiological environment by physical and chemical factors. Allowing the blood to stand (anaerobiosis), cooling, centrifuging, washing with salt solutions, dilution, substitutions for the normal plasma constituents, changes in pH, in  $O_2$  and  $CO_2$  tension, etc., must

be avoided. The error due to neglect of these precautions does not merely consist in the fact that the absolute metabolic values obtained are incorrect; respiration is also qualitatively changed, as for example, in its effect upon lactic acid fermentation or in the way it is influenced by HCN or CO. In dealing with sensitive cells, therefore, the metabolism must be measured in the unchanged blood, plasma, exudate, etc., though this makes the experiment more difficult, and as soon as possible after the cells are taken from the body.

The erythroblastic blood was taken in heparin (5 mgm. in 20 cc.) from the cubital vein, immediately saturated at  $37.5^\circ$  C. with a mixture of 5 per cent  $CO_2$  and 20 per cent  $O_2$ , shaken gently with glass beads for five minutes and filtered through gauze. Then, avoiding cooling of the blood, 6 cc., 3 cc. and 3 cc. respectively were pipetted into three Warburg manometer vessels (plain rectangular vessels with side bulbs of 17 cc. capacity) (1). The side bulbs of the vessels contained 0.2 cc. of an M/40 solution of lactic acid, the exact concentration of which was determined manometrically. While shaking at 150 oscillations per minute, the vessels in the thermostat were again saturated at  $37.5^\circ$  with a mixture of 5 per cent  $CO_2$  and 20 per cent  $O_2$ . Then the determination was begun in the aerobic manometer vessels 1 and 2. To obtain entirely anaerobic conditions vessel 3, containing 3 cc. of blood, was saturated for ten minutes with 2 liters of a mixture of 5 per cent  $CO_2$  in CO (2), since in erythrocytes a saturation with 5 per cent  $CO_2$  in nitrogen is not sufficient to produce complete anaerobiosis, because of the great amount of oxygen in combination with the hemoglobin. After the experiment the retention of lactic acid was determined (3) by tipping the 0.2 cc. lactic acid solution containing 112 c.mm. = 0.45 mgm. lactic acid, from the side bulb of the vessel into the blood. The retention of carbon dioxide was determined in a special vessel which contained 2 cc. of blood in the central space and 2 cc. of bicar-



bonate-Ringer solution in the outer space, by tipping the same amount of lactic acid into the bicarbonate-Ringer solution.

The metabolism, that is,  $O_2$  consumption,  $CO_2$  formation, aerobic and anaerobic lactic acid formation (glycolysis) was calculated according to the Warburg formulae (1). The dry weight was determined by Peschel's (4) method after centrifuging in special centrifuge tubes appropriate volumes of blood and drying at  $100^\circ C$ .

### RESULTS

Table I shows the metabolism of the blood of a normal six year old child and of the blood of the patient with erythroblastic anemia. Calculated per milligram of dry weight of cells, the respiration of the erythroblastic blood is 19 times as great, the anaerobic glycolysis 7 times as great and the rate of aerobic glycolysis about 4 times as great as in normal blood. The ratio of aerobic glycolysis to respiration is 2.3 in erythroblastic blood and 10 in normal blood. This means that not only are the absolute metabolic values of respiration and glycolysis considerably greater, but through the greater increase in respiration the ratio of splitting metabolism to respiration metabolism in the erythroblasts has shifted decidedly in favor of the respiration metabolism.

TABLE I

*Metabolism of erythroblastic blood and of normal blood*

	I $QO_2$ c.mm. oxygen consumed in 1 hour	II $QO_2^M$ c.mm. lactic acid formed under aerobic condi- tions in 1 hour*	III $QCO^M$ c.mm. lactic acid formed under anae- robic condi- tions in 1 hour*	IV Inhibi- tion of lactic acid forma- tion by oxygen	V Aerobic lactic acid forma- tion: respira- tion $\frac{II}{I}$
By 1 mgm. blood cells of the patient with cry- throblastic anemia	0.94	2.17	4.08	per cent 47	2.3
By 1 mgm. blood cells of a normal 6 year old child	0.05	0.5	0.58	13.8	10

\* 1 c.mm. = 0.004 mgm. lactic acid.

From the ratio of the metabolism figures of erythroblastic blood to those of normal blood, with the same number of leukocytes and thrombocytes one can obtain an approximate estimate

of the metabolism of the erythroblasts themselves as distinguished from the metabolism of normal red blood cells (Table II). It must be remembered, however, that blood containing numerous young non-nucleated erythrocytes has a greater respiration rate than normal blood, as Warburg (5) demonstrated in the blood of young rabbits and Morawitz et al. (6, 7) in the blood of anemic patients with marked blood regeneration.

TABLE II

*Calculation of the metabolism figures for 1 mgm. erythroblasts*

	I $QO_2$ c.mm. oxygen consumed in 1 hour	II $QO_2^M$ c.mm. lactic acid formed under aerobic condi- tions in 1 hour*	III $QCO^M$ c.mm. lactic acid formed under anae- robic condi- tions in 1 hour*	IV Inhibi- tion of lactic acid forma- tion by oxygen	V Aerobic lactic acid forma- tion: respira- tion $\frac{II}{I}$
1 mgm. blood cells of pa- tient with erythroblas- tic anemia (7 per cent nucleated red cells and 93 per cent normal blood cells) minus	0.940	2.17	4.08	per cent 47	2.3
0.93 mgm. normal blood cells (93 per cent of the cells of erythro- blastic blood)	0.047	0.47	0.54	13.8	10
0.07 mgm. erythroblasts (7 per cent of the cells of erythroblastic blood)	0.893	1.70	3.54		
1 mgm. erythroblasts	12.8	24.3	50.6	52	1.9

\* 1 c.mm. = 0.004 mgm. lactic acid.

Damblé (8) repeated the experiments of Morawitz with the newer Warburg methods, determining quantitatively the oxygen consumption of anemic blood in different forms of anemia and comparing it with the number of reticulocytes. In pernicious anemia and in secondary anemias with a non-reactive bone marrow he found the respiration of the erythrocytes less than in normal blood, whereas in secondary anemias with reactive bone marrow and high reticulocyte count the respiration was markedly increased. The highest value, an oxygen consumption for erythrocytes two and a half times that of normal blood, he found in a case of posthemorrhagic anemia in a young patient in the stage of blood regeneration, with a reticulocyte count of 10.2 per cent (erythrocytes 2.5 million, hemoglobin 51 per cent).

With a decreasing content of reticulocytes the respiration returned to normal.

The anaerobic glycolysis of the erythrocytes of anemic patients was quantitatively determined for the first time by W. Burger (2). He found the anaerobic lactic acid formation increased in about 45 per cent of his anemic patients compared with that of the erythrocytes of normal people. In one patient with pernicious anemia he found the anaerobic blood glycolysis increased four times. Unfortunately, counts for reticulocytes and for nucleated red blood cells are not given so that no relationship can be established between the number of nucleated red blood cells and reticulocytes and the glycolytic activity of the blood.

Since our patient, in contrast to most of the cases of erythroblastic anemia described in the literature (9, 10), showed no increase of reticulocytes in the blood, the reticulocyte count at the time of the experiment being below 1 per cent, we have no reason to use the metabolism figures of anemic blood for the metabolism of his non-nucleated blood corpuscles, but the calculations must be based on the metabolism figures of normal non-nucleated blood cells. The respiration of erythroblasts, however, is of such a different order of magnitude compared to the respiration of non-nucleated red blood cells, including those of regenerative anemic blood, that even if one used for basic figures twice the highest respiration rate found in anemias, with a large number of reticulocytes, the respiration value of the erythroblasts would not be appreciably decreased.

Table II shows the calculation of the metabolism figures for 0.07 mgm. of erythroblasts. In 1 mgm. of erythroblasts an oxygen consumption of 12.8 c.mm. per hour results, aerobic glycolysis of 24.3 c.mm. (=0.098 mgm. lactic acid) and anaerobic glycolysis of 50.6 c.mm. (=0.203 mgm. lactic acid). The ratio of respiration to aerobic glycolysis, which is 1:10 in normal blood, is here 1:1.9. If one applies the highest respiration rates found by Damblé in severe anemias with 10 per cent of the reticulocytes, instead of the respiration rates of normal non-nucleated blood cells and, as stated before, there is no reason to do this in the case of our patient since he has a normal reticulocyte count, one would find

a  $\text{QO}_2$  of 11.5 for the respiration of erythroblasts instead of a  $\text{QO}_2$  of 12.8. If one applies five times the respiration rate of normal blood cells which is twice the highest figure found in anemic blood, the respiration rate of erythroblasts would only be changed from 12.8 to 10.1.

The metabolism rates of human erythroblasts as compared with those of human leukocytes (4, 11) and of the nucleated red blood corpuscles of geese and alligators, which we examined by the same method at 37.5° C. in their own plasma are given in Table III. Similar values for the metabolism of blood cells of geese in bicarbonate-Ringer solution and for the oxygen consumption of alligator blood cells were found by Negelein

TABLE III  
*Metabolism of nucleated blood cells*

Cell species	I $\text{QO}_2$ c.mm. oxygen consumed in 1 hour	II $\text{QO}_2$ c.mm. lactic acid formed under aerobic condi- tions in 1 hour*	III $\text{QCO}_2$ c.mm. lactic acid formed under anae- robic condi- tions in 1 hour*	IV Inhibi- tion of lactic acid forma- tion by oxygen	V Aerobic lactic acid forma- tion: respira- tion II I
Human erythroblasts...	12.8	24.3	50.6	<i>per cent</i> 52	1.92
Human exudate leuko- cytes (11).....	22.8	16.8	57.8	71	0.74
Human leukemic lympho- cytes (4).....	5.8	0	11.1	100	0
Red blood cells of nor- mal geese.....	0.54	0	0.51	100	0
Red blood cells of ane- mic geese.....	1.06	0.16	1.9	92	0.15
Red blood cells of ali- gators.....	0.28	0.11	0.65	83	0.39

\* 1 c.mm. = 0.004 mgm. lactic acid.

(12) and Tipton (13). The respiration of human erythroblasts is seen to be 45 times greater than the respiration of the nucleated red blood corpuscles of alligators; about 24 times greater than the respiration of red blood cells of normal geese and about 12 times greater than the respiration of the erythroblasts of anemic geese. The metabolism of leukemic lymphocytes is considerably less than that of the erythroblastic cells. The ratio of aerobic glycolysis to respiration is in human erythroblasts 1.92, in human exudate leukocytes 0.74, in human leukemic leukocytes 0. The anaerobic glycolysis of human erythroblasts is 26 to 100 times greater than that of the nucleated red blood cells of geese and alligators and nearly

as great as the anaerobic glycolysis of leukocytes in inflammatory exudates.

In Table IV is given a comparison of the metabolism of erythroblasts with the metabolism of animal tissue cells. Human erythroblasts are among the cells with the highest anaerobic glycolysis, being of about the same order of magnitude as the glycolysis of embryonic tissue.

TABLE IV

*Metabolism figures for human and animal tissue cells*

Cell species	I QO <sub>2</sub> c.mm. oxygen consumed in 1 hour	II
		Q <sub>M</sub> <sup>N<sub>2</sub></sup> or CO c.mm. lactic acid formed under anaerobic conditions in 1 hour *
Human erythroblasts.....	12.8	50.6
Smooth muscle of human stomach (14).....	1.3	2.3
Adrenals of guinea pig (15).....	6	3
Human liver (embryo 4th month) (16).....	6.3	9.7
Human kidney (cortex) (16) (17)...	10	10.8
Human malignant tumor (1).....	5	28
Spermatozoa of steer (18).....	9	28
Rat embryo (0.9 to 3.1 mgm.) (19)	12	25
Embryonic chicken heart (4 days old) (20).....	30	52
Rat retina (21) (22).....	31	88

\* 1 c.mm. = 0.004 mgm. lactic acid.

## SUMMARY

The metabolism of human nucleated red blood cells has been measured manometrically in the blood of a patient with erythroblastic anemia. The erythroblasts show very high oxidative and fermentative metabolism. The respiration is approximately 200 times greater than that of normal non-nucleated human red blood cells (12.8:0.05), 100 times greater than the respiration of blood cells of anemic patients with a great number of reticulocytes (12.8:0.12), and about 20 times greater than that of the nucleated red blood cells of geese (12.8:0.54). In erythroblasts, as in human inflammatory exudates, in leukocytes and in non-nucleated red blood cells, respiration is not sufficient to cause the lactic acid formed to disappear, but the ratio of respiration metabolism to glycolytic metabolism as compared to that of non-nucleated red blood cells, is shifted decidedly in

favor of the respiration metabolism (1:1.9 as against 1:10).

The anaerobic lactic acid formation of erythroblasts is about 90 to 100 times greater than that of normal human blood cells and of normal erythrocytes of geese (50.6:0.58 and 0.51), and 25 times greater than that of erythrocytes of geese with marked anemia. The anaerobic lactic acid formation is of the same order of magnitude as that found in youngest embryonic tissue.

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# THE SALT AND WATER METABOLISM OF ADRENAL INSUFFICIENCY AND PARTIAL STARVATION IN RATS<sup>1</sup>

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All recent studies on the salt balance in adrenal insufficiency, both in the experimental animal and in Addison's disease, have shown that during this state sodium and chlorine are lost from the body (1, 2, 3, 4, 5). Regarding the potassium balance a difference of opinion has been expressed. Some observers have stressed a retention of potassium (3, 4), while our studies (5) demonstrated a loss of this salt. A possible explanation of this discrepancy may be found in the fact that the potassium balance is dependent to a great extent upon the food and water consumption during adrenal insufficiency, and that our rats show signs of anorexia earlier than is seen in the larger animals used by other workers. The present study was undertaken to demonstrate the effect of changes in the food consumption on the electrolytes and nitrogen balances of adrenalectomized rats.

Anorexia is an early symptom of insufficiency in the adrenalectomized rat. On the first day that sodium was added to the diet of the animals in adrenal insufficiency, the food intake returned to its original level, making it impossible to determine how much of the improvement in the salt balance was due to the added sodium and how much was due to the increased food consumption. For this reason it was found necessary, in an effort to evaluate the dietary factor, to conduct on normal nonadrenalectomized rats an experiment to show the influence on the salt and nitrogen balances of similar degrees of food restriction.

## PROCEDURES

The animals used in these experiments were young white rats of the Wistar strain with an average weight of approximately 75 to 150 grams at the onset of the study. Bilateral adrenalectomy was done in one stage. The operations were performed under light ether anesthesia, the

complete procedure taking only a few minutes. The animals evidenced no immediate ill effects from the operation and were active after a few moments.

The diet employed throughout was Colony Breeder's Diet with the following composition:

Whole ground wheat .....	660 grams
Whole ground corn .....	680 grams
Whole milk powder .....	420 grams
Linseed oil meal .....	140 grams
Alfalfa meal .....	40 grams
Liver .....	40 grams
CaCO <sub>3</sub> .....	10 grams
NaCl .....	10 grams
Dried Brewer's yeast .....	40 grams

Two solutions were used in the treatment of rats during insufficiency.

- (1) 0.9 per cent NaCl solution
- (2) Salt mixture solution  
    .7 per cent NaCl  
    .0329 per cent CaCl<sub>2</sub>  
    .0350 per cent KCl  
    .0150 per cent MgCl<sub>2</sub>·6H<sub>2</sub>O

Gamble metabolism cages were used for this study. In the majority of the adrenalectomy experiments the animals were studied in pairs, so as to increase the amount of material collected for study. In the undernutrition experiments, with the exception of Experiments XI and XII, only one rat was placed in each metabolism cage. This was necessary to make sure that each rat obtained the specified amount of food. In Experiments XI and XII glass partitions were placed in the cages separating the rats so that the individual rats obtained their allotted amounts of food.

A preservative was added to the urine and stool collecting receptacles. After completion of the study period, the cages and collecting apparatus were washed with glacial acetic acid and distilled water. Washings and urine collections were made up to volume for analyses. The stools and diet, before ashing, were finely powdered and dried.

## Wet ashing

Approximately one half the total volume of the urine collected was evaporated in quartz crucibles and digested with concentrated nitric acid under quartz covers on the steam bath for eighty hours or until the solution was clear. It was evaporated again to dryness, taken up in distilled water, and left on the steam bath another day so as to drive off nitric oxide. Ashing of the stools and diet required more prolonged digestion.

<sup>1</sup> Aided by a grant from the Faculty Research Committee of the University of Pennsylvania.

TABLE II  
Partial starvation experiments †

	Experiment number	Rat number	Duration	Water intake	Food intake	Urine output	Retentions						
							Ca	Mg	Na	K	P	Cl	N
Control periods	XI	76, 77	8	7.0	5.8	1.3	+21.7	- 3.2	+3.1	+15.4	+23.6	+ 3.1	+ 57.3
	XII	74, 75	11	6.5	5.8	0.9	+16.6	+ 1.6	+4.8	+21.9	+23.8	+ 3.9	+ 68.4
	XIII	102	4	13.0	9.6	7.8	+19.5	-20.4	+3.9	+ 1.8	+ 5.2	+10.7	+ 27.4
	XIV	122	4	16.0	10.0	5.8	+22.7	-15.9	+7.8	- 6.7	+ 6.4	+11.5	+162.8
	XV	123	4	18.0	13.6	6.0	+42.2	- 2.5	+3.9	+10.4	+19.1	+14.2	+166.7
	XVI	125	4	15.0	9.1	8.2	+24.1	- 7.6	+2.9	- 3.7	+ 8.2	+ 8.6	+ 30.4
	XVII	163	5	13.0	10.5	5.0	+37.1	+ 4.7	+9.0	+28.0	+18.3	+ 4.8	+110.5
	XVIII	164	5	11.0	10.8	2.6	+32.7	+ 1.2	+7.2	+20.8	+30.0	+ 5.6	+112.8
	XIX	165	5	14.0	7.6	4.4	+30.9	+ 5.9	+5.1	+19.3	+14.8	- 0.6	+ 70.4
	XX	166	5	13.6	10.2	3.4	+38.0	+ 3.0	+1.6	+21.3	+18.0	+ 2.6	+100.3
	XXI	167	3	21.0	12.7	3.0	+32.9	+ 6.3	+3.0	+ 9.0	+10.9	-25.2	+ 99.1
Averages				13.5	9.7	4.4	+28.1	- 2.4	+4.7	+12.5	+16.2	+ 3.6	+ 91.5
Partial starvation (food). Early periods	XI	76, 77	13	7.8	2.5	1.0	+ 2.8	- 0.6	+0.2	+ 4.7	+ 0.3	+ 1.3	+ 1.3
	XII	74, 75	9	4.4	4.7	0.6	+15.1	+ 1.6	-0.1	+ 8.6	-	+ 3.7	+ 41.3
	XIII	102	4	17.5	5.0	11.5	+ 1.6	- 4.5	+0.9	- 5.5	- 4.9	+ 1.8	- 30.5
	XIV	122	4	17.5	5.0	10.2	+ 5.3	- 5.4	-0.3	- 4.8	- 3.5	+ 5.8	+ 1.4
	XV	123	4	15.0	7.0	10.0	+ 8.8	- 4.8	+0.8	-11.8	- 1.9	+ 4.3	+ 59.7
	XVI	125	4	15.0	5.0	11.2	+14.2	- 4.5	+0.1	- 5.8	- 1.6	+ 3.5	- 15.8
Averages				12.9	4.9	7.4	+ 7.9	- 3.0	+0.3	- 2.4	- 2.1	+ 3.4	+ 57.5
Partial starvation (food). Late periods	XI	76, 77	8	6.3	1.3	4.3	+ 1.1	- 0.5	-4.5	- 9.4	- 3.6	- 0.9	- 40.5
	XII	74, 75	11	4.6	1.2	0.9	+ 1.0	- 1.0	-0.8	- 1.6	+ 3.1	+ 1.3	- 21.3
	XIII	102	5	8.4	2.5	4.4	+ 4.8	- 0.4	-0.4	- 2.3	+ 0.5	+ 4.8	- 10.5
	XIV	122	5	7.0	2.5	4.0	- 1.6	+ 0.3	-2.0	-10.2	- 2.1	- 0.8	+ 1.1
	XV	123	5	8.0	3.5	5.0	+ 7.2	- 0.7	-0.6	+ 5.5	- 9.6	+ 1.9	+ 16.6
	XVI	125	5	5.0	2.5	3.2	+ 3.9	- 0.9	-2.3	- 3.1	- 3.7	+ 3.6	- 43.6
Averages				6.5	2.3	3.6	+ 2.7	- 0.5	- 1.8	- 3.5	- 2.6	+ 1.9	- 16.4
Partial starvation (water and food). Early periods	XVII	163	5	6.0	5.5	2.4	+24.0	0.0	-0.8	+ 3.2	+ 2.6	- 0.5	+ 18.7
	XVIII	164	5	6.0	5.5	1.6	+19.8	- 0.6	0.0	- 1.3	+ 4.2	- 4.2	+ 23.5
	XIX	165	5	6.0	4.0	1.6	+11.1	+ 0.6	+3.1	+ 9.4	+ 3.4	+ 2.7	+ 15.3
	XX	166	5	6.0	5.5	1.6	+16.1	+ 2.9	-0.7	+ 7.1	+ 4.5	+ 0.5	+ 15.7
	XXI	167	5	6.0	6.0	1.8	+18.1	+ 3.9	+0.3	+ 9.4	+ 5.0	+ 1.9	+ 36.4
Averages				6.0	5.3	1.8	+17.8	+ 1.4	+0.5	+ 5.6	+ 3.9	+0.08	+ 21.9
Partial starvation (water and food). Late periods	XVII	163	5	3.0	3.0	1.2	+ 6.7	- 0.4	-2.4	-18.8	- 3.0	- 4.5	- 50.0
	XVIII	164	5	3.0	3.0	1.0	+ 0.9	- 0.6	-0.1	-15.5	- 2.8	- 2.5	- 29.4
	XIX	165	5	2.0	2.0	1.2	+ 5.6	- 2.8	-3.4	- 9.0	- 4.9	- 5.3	- 20.0
	XX	166	5	3.0	3.0	1.0	+ 8.1	- 1.8	-5.3	- 3.5	- 1.9	- 0.1	- 25.9
	XXI	167	5	3.0	3.0	1.4	+ 6.8	-	-3.6	-17.3	- 2.4	- 3.8	- 35.9
Averages				2.8	2.8	1.2	+ 5.6	- 1.4	-2.9	-12.8	- 3.0	- 3.2	- 32.2

† All values per rat per day.

one-half the normal amount. After several days, during the stage of more advanced insufficiency, the food intake drops again to one-fourth the normal, and then just prior to death, food is entirely refused. In Experiments XVII through XXI, where the water as well as the food intake was restricted, the balances were similar to those where food alone was restricted, except for the late period of water and food restriction. Here, with the marked decrease in the water intake, the negative potassium and nitrogen balances were

greater, demonstrating more tissue destruction. In Experiments XI and XII data are given from a different investigation on undernutrition where the food restrictions of normal rats paralleled the decreasing food consumption of animals in vitamin B<sub>1</sub> deficiency. In these experiments water was allowed *ad libitum*.

The results of these underfeeding experiments, as given in Table II, demonstrate that during undernutrition the changes in the retentions of the elements investigated proceed in a similar direc-

tion to those of adrenal insufficiency, but are of a lesser magnitude. In the adrenal insufficiency experiments the average daily balance during the control periods for sodium was  $+9$  mgm., for potassium  $+13.8$  mgm. During insufficiency when the average food consumption was about one-third normal, the average daily balance of sodium was  $-4.7$  mgm., and of potassium  $-14.8$  mgm. In the partial starvation experiments the average daily balance during the control period was  $+4.7$  mgm. for sodium and  $+12.5$  mgm. for potassium, while during the period where the food intake was one-fourth normal, the average daily sodium balance was  $-1.8$  mgm., and the average daily potassium balance was  $-3.5$  mgm. The chlorine losses were also greater during adrenal insufficiency than during partial starvation. The losses of calcium, magnesium, and phosphorus during the two situations are essentially comparable. The differences in the average nitrogen retentions of these two experiments, as given in Tables I and II, might depend on the fact that during the early postoperative periods some of the animals did not develop a severe enough insufficiency to have a marked anorexia and a negative nitrogen balance as in Experiment 7. A false average value for the adrenalectomized rats would therefore be obtained. In the late postoperative periods kidney failure might prevent the nitrogen excretion. *It would seem, therefore, that while the decreased food consumption during advanced adrenal insufficiency is in a large part responsible for the altered electrolyte and nitrogen balances, the adrenal gland per se partly influences the balances of those electrolytes which are associated with the water of the body, namely: sodium, potassium, and chlorine.*

#### C. Treatment with salt solutions

Doubt has been expressed by some workers as to whether completely adrenalectomized rats will respond to salt therapy. The degree of insufficiency obtained by the type of operation performed in our laboratory is fatal to the animals in 80 per cent of the untreated cases. Nevertheless, salt therapy is successful in rats with this degree of insufficiency. Table I, Experiments 4, 5, 6, 8 and 9, shows the beneficial effect of salt therapy on the electrolyte and nitrogen balances

following adrenalectomy. In each instance following salt therapy there was a return of the electrolyte and nitrogen balances to normal. This therapy, it is to be stressed, was always associated with an immediate return of the appetite. It should be pointed out here that salt therapy may be unsuccessful in restoring rats when adrenalectomy is performed at a very early age.

The weight curves of the adrenalectomized animals on both the salt mixture solution and the normal saline solution demonstrate that the growth of these animals is not as great as that of normal controls. We have, however, kept completely adrenalectomized animals in apparently good health for over ten months by giving them either one of these two salt solutions. On the other hand, animals treated with either of these salt solutions eventually die in insufficiency, demonstrating that the salts given do not completely restore the body equilibrium. This suggests that other body mechanisms disturbed by adrenalectomy in rats are not restored when the animals are given these additional salts. This is in keeping with clinical experience in the use of sodium chloride in Addison's disease.

#### D. Water balance

Since adrenalectomized animals treated by the addition of salt to their drinking water consume large amounts of this fluid, the question arises whether these animals drink abnormally large quantities of salt solution in order to obtain excess water or the added salt. That adrenalectomized animals soon refuse to drink distilled water is good evidence, we believe, that additional salt is desired. In an effort to determine whether the actual amount of salt ingested was the important factor, we used varying concentrations of sodium chloride in the water. In one group of rats the average hourly consumption of fluid per rat on the 0.65 per cent sodium chloride solution was 3.6 cc., and on the 1.4 per cent solution it was 1.4 cc. It is interesting to find that the rats on the weaker solution drank enough of it to keep their salt intake at a level comparable with that of the rats on the more concentrated salt solution. Adrenalectomized rats given the added salt in their diet but deprived of water died much more quickly than normal rats deprived of water.





occurred when the added salt was removed from the drinking water, demonstrating the ability of normal rats to regulate their salt retentions. We would have expected a sudden weight loss when salt was withdrawn from the drinking water of the normal rats if the previously added salt was being retained with water in the body. However, it is well known that the normal kidney can excrete a large excess of salt. The fact that the adrenalectomized animals, which were gaining weight while on the added salt, immediately lost weight when the salt was withdrawn, demonstrates that these adrenalectomized rats had not stored in the body any salt that might tide them over for even short periods of salt deprivation, and were unable to regulate their salt and water retentions. It is, however, apparent that adrenalectomized animals have not completely lost their power to regulate their salt and water balance when adequate amounts of these substances are administered.

When the figures for salt balances are calculated so as to compare the milligrams of each element excreted to the grams of food consumed, it becomes obvious that during adrenal insufficiency the animal does not conserve its salts. For example, in Experiment 2, during the control period the animal excreted 2.2 mgm. calcium for each gram of food consumed, while during insufficiency, despite the lowered food intake, the animal excreted 3.2 mgm. calcium per gram of food consumed. For magnesium the figure for the control period is 1.4 against 1.6 for the insufficient period, and so with sodium where the figures are 2.2 as compared to 3.0. For potassium we have 5.1 to 9.1; for phosphorus 2.3 to 3.3; for chlorine 4.3 to 5.1; and for nitrogen 19.5 to 23.5. In each of the experiments this loss of salt economy during adrenal insufficiency is demonstrated. A similar lack of salt conservation has been found in normal rats which are partially starved. *Thus, it is again demonstrated that the electrolyte changes occurring in the organism during adrenal insufficiency are influenced by the lowered food intake.*

#### DISCUSSION

##### *1. Influence of food consumption on electrolyte and nitrogen metabolism*

The similarity of the altered electrolyte and nitrogen balances during partial starvation and

during adrenal insufficiency indicates, as was suggested in our earlier paper (5), that the changes in electrolyte and nitrogen balance found during moderately advanced adrenal insufficiency are in a measure dependent upon the lowered food consumption after adrenalectomy. Harrop and his associates (14) have shown that the lowered blood sugar found during adrenal insufficiency in dogs is dependent upon the lowered food intake. However, it is apparent from our data that the lowered food intake is not the entire cause of the altered balances, for the reduced retentions of sodium, potassium, and chlorine during adrenal insufficiency are greater than those seen in similar degrees of undernutrition in normal rats. It thus seems clear that the adrenal gland itself has some influence on the retentions of those electrolytes associated with the body water. In agreement with this, Loeb, Harrop, and their coworkers (3, 4) have demonstrated alterations in the sodium balance prior to the appearance of anorexia. Regarding the calcium, magnesium, phosphorus, and nitrogen, the retentions of these elements in adrenal insufficiency parallel the amount of food consumed.

##### *2. Sodium metabolism*

Regarding the influence of the adrenal gland on the sodium metabolism, it would appear that with the removal of this gland the following sequence of events occurs. Initially there is a diuresis. Since the extracellular water is the most available, sodium, which is the chief electrolyte of this body fluid compartment, is the first salt to be excreted. As the store of extracellular water with its sodium is depleted, the intracellular water with its potassium is next drawn upon to keep up a falling circulating fluid volume. There seems to be no doubt that sodium is lost from the body during the dehydration of adrenal insufficiency. It is also true that during undernutrition sodium is lost, but to a lesser extent. Similarly, in many unrelated conditions which have dehydration as a factor in common, i.e., starvation (15), diabetic acidosis, diuresis (16, 17), and nephritis (18), sodium is lost from the body in large amounts, and the sodium and chlorine changes of the blood serum, where they have been investigated, are similar to the sodium and chlorine changes of the blood in adrenal insufficiency. From these facts

alone it would appear that the sodium loss in adrenal insufficiency is not necessarily a specific function of the adrenal gland but is an accompaniment of dehydration. That during adrenal insufficiency the sodium concentration in the urine is increased despite the diuresis (3), demonstrating a loss of sodium in amounts above that expected from the water loss, does not *ipso facto* imply that sodium is selectively excreted. The extra water which should have been excreted by the kidney, if there had been simply a diuresis of extracellular water, may have been partly lost through the lungs or lost into the body cells. Darrow and Yannet (19) have shown that when there is a large loss of sodium from the extracellular water, in the redistribution of water, water enters the cells to reestablish an osmotic equilibrium. This must occur prior to the loss of potassium from the cells. The kidney in this case excretes a more concentrated sodium solution so as to permit the water retention. In a study by Kerpel-Fronius and Butler (17) where diuretin was administered to rabbits, they found that along with a marked diuresis, sodium and potassium were lost in large quantities. The blood sodium in these animals was found to be lowered to a degree comparable with that of adrenal insufficiency. In their Experiment 4, on the first diuretin day, the concentration of sodium in the urine was about twice that of the sodium concentration on the next day when diuretin was not used. This increased concentration of sodium in the urine with an increased urinary volume is similar to the findings of Loeb et al. (3) in adrenal insufficiency.

That the addition of sodium chloride to the intake during insufficiency restores the animal is again not indicative of a specific regulation of the sodium metabolism *per se* by the adrenal glands. It merely demonstrates that replacement of sodium is specific for sodium loss whether it is due to adrenal insufficiency, nephritis, or diarrhea. In all forms of shock due to dehydration sodium chloride solution aids greatly in restoration to normal. In the dehydration due to severe diarrhea in infancy, sodium chloride solution is beneficial.

The recent studies of Swingle and his associates (20) have demonstrated that with hormonal therapy during starvation clinical improvement in

the adrenalectomized animal may occur prior to any change in the lowered blood sodium.

It is most likely that the beneficial effect of sodium when given in large amounts results from its ability, when the animal has access to water, to restore the depleted water of the blood and interstitial tissue which had been lost during the early diuresis. Finally, sodium replacement therapy has not completely restored the animal or the human in adrenal insufficiency. Several of our rats, more especially the very young, have died of insufficiency while on sodium.

### 3. Potassium metabolism

*A negative potassium balance was demonstrated in each instance during adrenal insufficiency in our rats.* The negative balance for this element was greater than that found in similar degrees of partial starvation in the normal rat. However, when this partial starvation of food was associated with a forced reduction in the water intake, the negative potassium balances were comparable to those of adrenal insufficiency. In the former circumstance, the forced reduction of water most probably resulted in an increased amount of tissue destruction, thus liberating more potassium for excretion.

In many instances during insufficiency the potassium losses were four or five times greater than those which should have been expected if potassium were lost only on cell destruction, as calculated by the nitrogen excretion. Loeb and his co-workers (3) state that potassium is retained during adrenal insufficiency, yet in only one of the three adrenalectomized dogs in their experiment was there a positive potassium balance. It is to be stressed that when this dog was in a positive potassium balance anorexia was not present. However, when food was refused by this dog, as in the case of the other two dogs, and insufficiency progressed, this animal also developed a negative potassium balance. Another interesting study on potassium balance during adrenal insufficiency was that performed by Harrop and his co-workers (21). These workers maintain that during insufficiency there is a retention of potassium, and after adrenal cortical hormone is administered to the animal the potassium balance becomes negative. Again, when the potassium balance is posi-

tive in the dog reported by these authors, the animal is consuming close to his normal food intake. However, when the potassium balance is negative, the animal has developed a marked anorexia, in fact complete starvation. Starvation, as Gamble and his coworkers (15) found in their study of a child fasting twenty-four hours, resulted in the excretion of about eight times as much intracellular water, measured by the potassium excretion, as that of extracellular water, measured by the sodium excretion. The changes in the potassium balance ascribed by Harrop et al. (21) to an effect of the withdrawal and the addition of the cortical hormone may be due to the fact that the animal had not yet shown evidence of severe adrenal insufficiency during the short period of hormone deprivation and secondly, the changes observed following the administration of hormone may be due in part to a lag in the return of the appetite and the electrolyte balance to normal. Furthermore, during the period of deprivation of hormone the elevated blood potassium is associated with an elevated blood urea, and thus may be dependent on kidney insufficiency. When hormone is given, the excretion of the large amount of potassium is associated with the fall in blood urea indicating an improved kidney function. This improvement in kidney function which permits excretion of the once retained potassium might depend on extrarenal factors. Swingle and his associates (22) have demonstrated that hormone given to adrenalectomized animals deprived of food and water produces a temporary rise in the blood pressure, a return towards normal of the blood volume, and a lowering of the blood urea. This effect of the hormone may be one aside from its so-called water and salt regulation and may be operative through its ability to combat the shock-like symptoms of adrenal insufficiency.

The apparent difference between the findings of Loeb, Harrop, and their coworkers and those that we present in this paper may be dependent upon the fact that adrenalectomized rats develop anorexia and severe insufficiency with its dehydration more rapidly than do the larger animals (dogs) used by these above-mentioned workers.

It is questionable whether the elevated blood potassium found during adrenal insufficiency by Harrop and his coworkers indicates that a positive potassium balance occurs during this disturbance.

This may be analogous to the situation of calcium in hyperparathyroidism, where an elevated blood calcium exists while the body is actually being depleted of its calcium. Nevertheless, how can the elevated blood potassium be explained? In those instances where a high blood potassium has been reported, it has always been associated with a high blood urea nitrogen. This piling up of urea in the blood, a condition which comes on after insufficiency is moderately advanced, is generally believed to be due to an inadequate supply of water for renal excretion which in turn reduces the kidney blood flow. (The water intake of the animals with adrenal insufficiency is reduced.) Schoenthal and his coworkers (23) found the urea clearance to be greatly reduced in dehydrated infants. All this might indicate that when the blood potassium is elevated in adrenal insufficiency, there is already a functional kidney insufficiency.

Thus it seems likely that with the continued tissue destruction due to dehydration and inanition resulting in an increasing liberation of urea and potassium for excretion, and with the progressively decreasing functional activity of the kidney, also due in part to the dehydration, there exists an adequate explanation for the elevated blood potassium.

The large loss of potassium found in our animals must have occurred prior to this kidney insufficiency.

In support of this hypothesis is the fact that the blood potassium is elevated in certain instances of kidney damage (18). Further substantiation has been produced by Butler (24), who has found the blood serum potassium elevated in dehydration. The high serum potassium was associated with an elevated blood urea and a decreased blood sodium and chlorine, findings similar to those of adrenal insufficiency.

In a small group of adrenalectomized rats where potassium chloride instead of sodium chloride was given in the drinking water, the animals died rapidly. This apparent toxic effect of potassium on the adrenalectomized rats may be due either to its dehydrating action resulting in a further diuresis or possibly to an ill effect of the high blood potassium on some organ or physiological mechanism. In fact, both of these processes are probably operative. Furthermore, the suscepti-

bility of the adrenalectomized animals to various toxins is well established.

Findings strikingly similar to those of adrenal insufficiency have been produced by Kerpel-Fronius (16) in the dehydration of normal rabbits brought about by salt restriction and urea feedings. In the dehydration which he produced by salt-poor feeding (sodium chloride), there occurred a loss of sodium from the body in association with a normal potassium retention. This parallels the findings in mild or early adrenal insufficiency as demonstrated by Harrop et al. (21). In the more severe dehydration, which Kerpel-Fronius produced by feeding urea, the changes were similar to those found by us in the dehydration due to advanced adrenal insufficiency where a negative balance of both sodium and potassium occurs. Since the weight loss (water loss) in the urea experiment greatly exceeded that of the sodium chloride free experiment, *it would appear that the severity of salt losses and whether or not potassium is excessively excreted depends upon the degree of dehydration.*

#### 4. Nitrogen metabolism

From the data presented here it would appear that the negative nitrogen balance seen during adrenal insufficiency in our rats is in a large measure dependent upon the decreased food consumption. In addition, as a result of the dehydration, tissue destruction with its release of nitrogen also occurs. From the experimental data of Loeb, Harrop, and their coworkers (3, 4), it is seen that their animals were also in a negative nitrogen balance when they were not consuming much food.

That an animal may be in negative nitrogen balance while his blood urea is elevated is seen from the experiments of McCance (25). The elevated blood urea is a manifestation of two factors, first, excessive tissue breakdown, and second, inadequate urea excretion due to the dehydration with its decreased kidney blood flow and subsequent kidney insufficiency.

It should be stressed that when urea is liberated in excessive amounts from the cells during the tissue destruction in adrenal insufficiency, it is in itself a mechanism for further dehydration through diuresis.

#### CONCLUSIONS

Following adrenalectomy there occurs early a marked diuresis associated with decreased consumption of food and water producing dehydration and undernutrition, and resulting in an excessive loss of sodium, potassium, and chlorine, along with the other elements investigated. Sodium and extracellular water are lost first, followed, as insufficiency progresses, by a loss of potassium and intracellular water. It has been demonstrated that the partial starvation (water and food) of adrenal insufficiency alone plays a major rôle in lowering the retentions of these electrolytes and nitrogen during adrenal insufficiency.

There seems to be no definite indication that either sodium or potassium is selectively regulated by the adrenal gland. Instead it would appear that the water and general electrolyte metabolism is affected by this gland. In a broad sense, the function of the adrenal gland cortex regarding this one phase, salt and water regulation, appears to be of an antidiuretic nature, since its removal precipitates a diuresis, the underlying factor for so many of the findings in this deficiency.

The improvement of the adrenalectomized animal after cortical hormone therapy and prior to the restoration of the salt and water imbalance (20), and the failure of salt therapy to be uniformly successful in the adrenalectomized rat suggest the existence of other important functions for the cortical hormone. The ability of the cortical hormone to temporarily raise the blood pressure, lower the elevated blood urea, and restore the blood volume towards normal, when injected into animals with adrenal insufficiency on food and water starvation, as was demonstrated by Swingle and his coworkers (22), might account in part for its beneficial effect on the kidney excretion in the presence of an unchanging intake of water and salt.

The salt imbalances found during adrenal insufficiency are similar to those found in other instances of severe dehydration and starvation.

#### SUMMARY

1. Balance studies have been made for sodium, potassium, calcium, magnesium, chlorine, phosphorus, and nitrogen on rats before and after

adrenalectomy, and also following salt therapy.

2. Balance studies were made for similar elements on rats during partial starvation.

3. Adrenal insufficiency was associated with the loss from the body of all the elements investigated.

4. The salt balances in partial starvation were similar to those occurring during adrenal insufficiency, but of a lesser magnitude.

5. At the same level of food and water intake the losses of sodium, potassium, and chlorine were much larger during adrenal insufficiency than during partial starvation, indicating that the electrolyte losses during adrenal insufficiency are not entirely due to the lowered consumption of food.

6. It is suggested that the cortex of the adrenal gland influences the general electrolyte and water balances, not specifically the sodium or potassium balances.

7. It has been demonstrated that salt treatment is of value but does not completely restore the adrenalectomized rat, which suggests, as others have maintained from different evidence, the existence of other functions for the adrenal cortex.

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# INTRACELLULAR FLUID LOSS IN HEMORRHAGE

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Following a sublethal loss of blood an immediate inflow of fluid into the capillary bed takes place. This fact has been repeatedly demonstrated by measurements of reduction in the concentration of plasma protein, fall in specific gravity of the plasma (1), and diminution in dry residue of serum (2). There seems to be no clear evidence regarding the sources of the fluid by which plasma volume is restored after hemorrhage, the usual statement merely implicating "the tissues." On the other hand, the importance of the splenic reservoir as a source of formed elements after loss of blood is well known.

Attempts to obtain information on this subject by tissue analyses following hemorrhage have on the whole been inconclusive (3), owing to technical difficulties, variation in composition of different tissues, and the small changes to be expected when the volume of blood lost is considered in relation to the body mass.

In one of the first careful studies of base metabolism Gamble introduced the principle, now widely accepted, of the parallel movement of base and water in the body (4). Since potassium is the chief base in cell fluid and since the concentration of potassium outside cells is kept rigorously low, by measuring the amount of potassium excreted from the body above the intake and above that liberated by protoplasmic breakdown a measurement of the extent of fluid lost from cells by diffusion is obtained. By using this principle Butler, McKhann and Gamble were able to compute the loss of intracellular fluid in diarrheal disease of infants (5).

The present study was directed at determining whether the fluid which supports plasma volume under the exigencies of hemorrhage is entirely of extracellular origin, or whether intracellular fluid as well is drawn into the circulating blood.

## PLAN OF EXPERIMENT

Large healthy female dogs of docile disposition were fasted in metabolism cages. The animals were allowed to drink distilled water as desired. Daily determinations were made of weight, rectal temperature and water drunk. Daily catheterization was performed, and usually the animals did not void between catheterizations. After a basal fasting level of excretion of electrolyte in urine was established, which usually required four or five days, the dogs were bled from jugular vein or femoral artery to the extent of 2 to 3.5 per cent of body weight in a single hemorrhage lasting five to twenty minutes. No anesthesia was used except local injection of a small amount of novocaine in case blood was taken from the femoral artery. When the femoral artery was used a point was selected sufficiently peripheral to exclude the factor of anoxemia in the extremity, for, as Baetjer has shown, severe anoxemia leads to diffusion of potassium from the tissues of an extremity into the plasma (6). Following the hemorrhage the animal was not allowed to drink for at least three hours, after which water was given freely. The observations were continued for three to four days after the hemorrhage. Blood samples were taken at appropriate intervals.

An important feature of the experimental plan consisted in conducting the study on the basis of fasting metabolism. In this way the difficulties and inaccuracies of measurement of the electrolyte intake in the food and excretion in the feces were avoided.

Standard analytical procedures were used. Sodium of serum and urine was determined by the gravimetric technique of Butler and Tuthill (13); potassium of serum and urine by Fiske's modified cobaltinitrite method in which potassium is reprecipitated as potassium acid tartrate (14); chloride by Wilson and Ball's method of wet ashing with nitric acid and potassium permanganate and Volhard titration (15); carbon dioxide content of the serum according to Van Slyke and Sendroy (16); total nitrogen by macro-Kjeldahl (17); nonprotein nitrogen of serum by micro-digestion and nesslerization (18). Hematocrit readings were made by heparinization of a sample of blood, followed by centrifuging in hematocrit tubes at 2000 r.p.m. until no further change in volume was perceptible, precautions being taken against loss of CO<sub>2</sub>.

## PRESENTATION OF DATA

In Figure 1 are shown the daily excretion of sodium, potassium, chloride, and total nitrogen and daily urine volume and water drunk, before and

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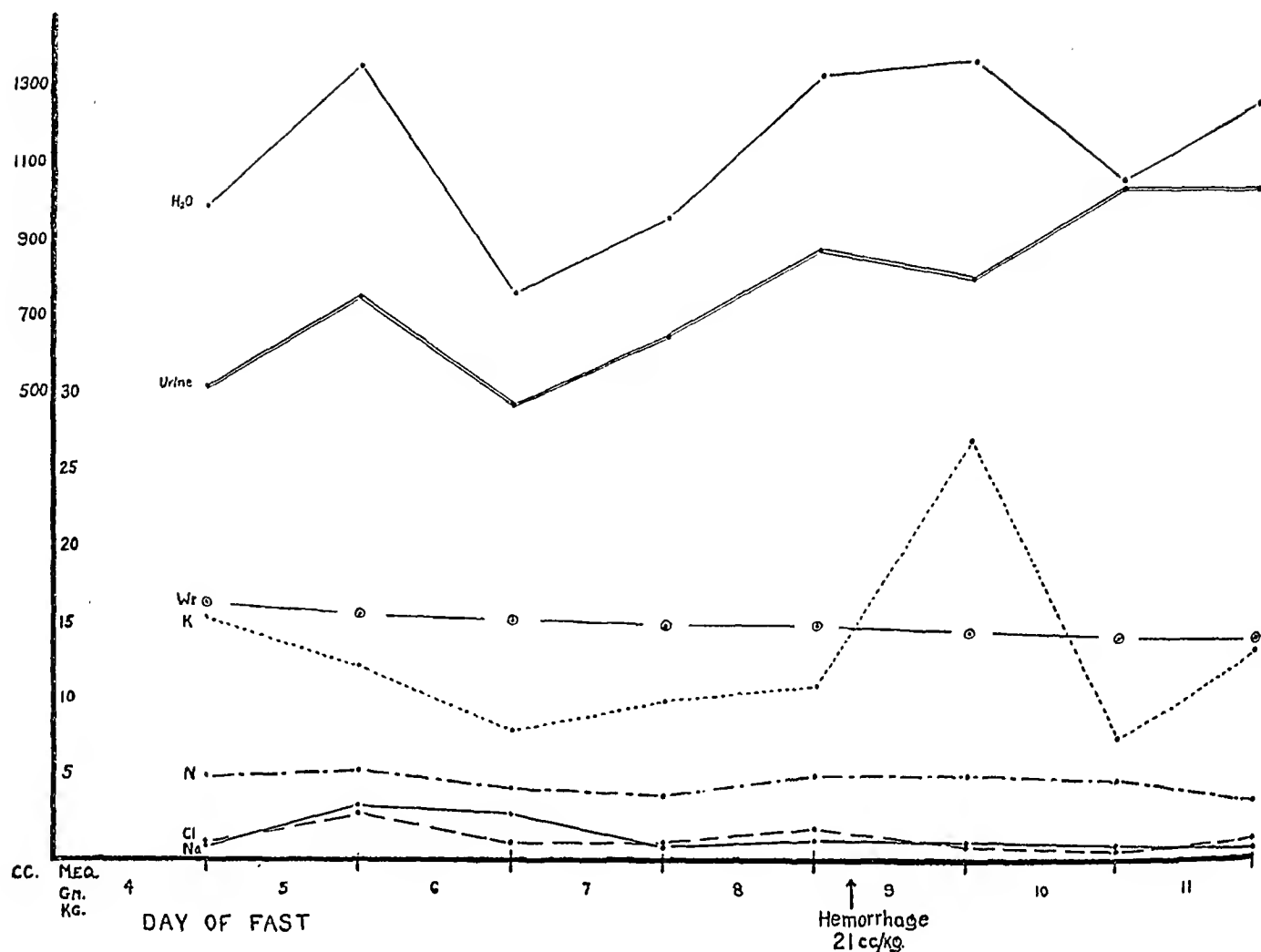


FIG. 1. EXCRETION DATA, EXPERIMENT 36-23.

after hemorrhage. It is to be noted that by the fifth day of fasting a basal level of excretion has been reached, although a gradual decrease in the quantity of electrolyte and nitrogen excreted daily is evident. The marked increase in excretion of the intracellular base, potassium, and the lack of change in excretion of sodium and chloride following hemorrhage are clearly shown. The increase in urinary potassium occurs only

within the twenty-four hour interval after hemorrhage. No appreciable change in the elimination of nitrogen takes place.

In Table I are shown the quantities of potassium and nitrogen excreted in the urine before and after hemorrhage. The daily basal level of excretion is to be compared with the amount excreted in the twenty-four hours following hemorrhage. The much greater increase in potassium

TABLE I  
Data on potassium and nitrogen excretion

Experiment number	Weight	Hemorrhage	K excretion		N excretion		K : N ratio	
			Basal	Posthemorrhage	Basal	Posthemorrhage	Basal	Posthemorrhage
	kgm.	cc. per kgm.	m.eq. per day	m.eq. per day	grams per day	grams per day		
36-12	20.15	22	12.5	31.0	4.1	5.8	3.0	5.3
36-13	18.70	26	11.5	22.5	5.2	5.2	2.2	4.3
36-17	12.30	36	12.5	24.8	3.1	4.6	4.0	5.4
36-23	15.40	21	11.1	28.1	5.4	5.4	2.1	5.2
36-25	15.00	31	10.4	26.4	3.4	4.6	3.1	5.7

TABLE II

*Experiment 36-25. Excretion data and blood values before hemorrhage, after hemorrhage but before ingestion of water, and after ingestion of water*

Day of fast	Time	Weight		H <sub>2</sub> O drunk	Serum values					Hematocrit	Urine. Quantities excreted				
					Na	K	Cl	Serum protein	N.P.N.		Volume	K	Na	N	Cl
		kgm.		cc.	m.eq. per liter	m.eq. per liter	m.eq. per liter	grams per 100 cc.	mgm. per cent	per cent	cc.	m.eq.	m.eq.	grams	m.eq.
5	11:45 a.m.	15.00	Bladder emptied	0											
6	9:20 a.m.	15.00	Bladder emptied	500	146.4	6.0	112.0	5.85	30.0	45.6	145	9.52	0.4	3.12	1.4
	11:50 a.m.		Hemorrhage	0											
			470 cc.												
7	10:00 p.m.		Bladder emptied	0	142.1	5.7	112.0	5.50	21.9	43.0	56	19.25	1.8	2.03	3.1
	11:00 p.m.		Given water												
	9:50 a.m.	14.35	Bladder emptied	755	139.7	5.2	109.0	5.29	21.7	36.8	400	7.12	2.1	2.60	3.8

as compared with nitrogen and consequent rise in potassium: nitrogen ratio is clearly shown.

From Table II, it will be seen that during the twenty-two hour period before hemorrhage the dog drank 500 cc. water, excreted 145 cc. urine and 9.52 m.eq. of potassium. In the twelve hours (roughly) after hemorrhage no water was taken, 56 cc. urine were excreted containing 19.25 m.eq. of potassium, representing over a fourfold increase in rate of excretion of potassium. The animal was then allowed to drink and took 755 cc. water during the following twelve hours. In this period potassium excretion fell off sharply. This experiment was undertaken to show that following hemorrhage the loss of potassium from cells takes place immediately after the loss of blood and not chiefly during the subsequent period when water is drunk. Note the reduction in serum sodium, potassium, nonprotein nitrogen and protein before water is taken, with chloride concentration remaining unchanged.

The data presented in Table III were obtained

TABLE III

*Serum concentrations before and after hemorrhage*

Experiment number	Hemorrhage	Na	K	Cl	CO <sub>2</sub>	N.P.N.	Serum protein
	cc. per kgm.	m.eq. per liter	m.eq. per liter	m.eq. per liter	volume per cent	mgm. per cent	grams per cent
36-12	22	144.5	4.5	105.0	56.7	22.7	5.9
		143.0	4.2	107.0	55.1	23.1	5.2
36-13	26	146.0	4.7	107.0	54.6	23.1	6.6
		139.0	4.6	103.0	49.8	28.6	6.1
36-17	36	144.6	5.0	112.5	50.5	23.5	5.7
		143.5	4.6	107.5	52.7	25.2	4.6
36-23	21	146.8	5.3	106.0		23.7	6.7
		136.9	5.0	103.5		30.8	6.0
36-25	31	146.4	6.0	112.0		30.0	5.9
		139.7	5.2	109.0		21.7	5.3

in blood samples taken just before hemorrhage and again twenty-four hours later, the animal having been allowed to drink water freely following an interval of at least three hours after hemorrhage. Serum sodium concentration is invariably reduced, while serum chloride in one instance is increased. CO<sub>2</sub> and nonprotein nitrogen show no significant change, while serum protein is invariably reduced. The concentration of serum potassium likewise always falls.

#### DISCUSSION

From the data presented it is quite clear that following hemorrhage of the extent produced in these experiments there is a marked increase in excretion of potassium in the urine. Concerning this loss of potassium from the cells two explanations come to mind, either an increase in breakdown of cell protoplasm after hemorrhage, or a simple diffusion of fluid from the cell with reduction in cell volume. Were the potassium liberated from the cells by a process of disorganization of cell protoplasm then there should be an equivalent liberation of nitrogen. By examination of Table I one sees that such is not the case, since the potassium excreted after hemorrhage doubled or trebled, while the urinary nitrogen showed slight or no increase. Hence there is a sharp rise in the potassium to nitrogen ratios. These facts have a clear significance since the maintenance of normal or even reduced concentrations of serum potassium and nonprotein nitrogen following hemorrhage (Table III), excludes an abnormal retention of these elements.

The possibility of a relationship between the water ingested by the animal and the observed

dilution of the blood plasma and loss of intracellular electrolyte must be considered. It might, for instance, be suggested that a reduction of the total osmotic value of extracellular fluid by retention of ingested water would make necessary a removal of intracellular electrolyte in order to produce osmotic equivalence without a change in the volume of intracellular water. In the presence of such an adjustment, loss of potassium would not be accompanied by a removal of cell water.

To investigate this question the time relationships in the excretion of potassium following hemorrhage were studied. From the data obtained in Experiment 36-25 and tabulated in Table II it is clear that cell potassium was lost largely during the period of adjustment immediately following hemorrhage and before water was ingested. In this experiment the rate of elimination of potassium in the urine in the twelve hour period after hemorrhage during which no water was taken was over four times as great as during the twelve hour period before hemorrhage. Therefore, the posthemorrhagic loss of potassium from cells is not the effect of osmotic adjustments occasioned by retention of ingested water.

The mechanism of dilution of plasma following hemorrhage can dependably be viewed in the light of Starling's theory (11, 12), of counterpoised hydrostatic pressure and colloid osmotic force of plasma proteins in the capillary bed, for the fundamental validity of this theory has remained unchallenged. With reduction of capillary hydrostatic pressure consequent on hemorrhage and favored by activation of the sympathoadrenal apparatus (7), the unopposed colloid osmotic pressure of the plasma proteins becomes effective in creating a flow of fluid into the capillary bed. From the evidence obtained in these experiments it is clear that movement of fluid out of the cells as well as interstitial areas takes place.

Let us consider this fact further. We are accustomed to regard interstitial fluid as an extensive reservoir from which plasma losses can be readily replaced, its electrolyte composition being so nearly identical with that of the plasma. That water and the unsuitable electrolyte, potassium, should also be immediately withdrawn from tissue cells would not, on teleological grounds, be

expected. We must remember, however, that the expectation that plasma losses will be replaced exclusively by interstitial fluid rests on the assumption that there is everywhere in the tissues an appreciable quantity of interstitial fluid between the capillaries and tissue cells. It will be admitted that this premise has not been established to the extent of excluding the possibility that capillary wall may often lie in contiguity with cell wall.

On this basis we venture an explanation of the entrance of cell fluid into the capillaries. One visualizes two sets of colloid osmotic forces in the tissues, one centered within the capillary and created by plasma protein, the other centered within the cell and incident to the high intracellular content of protein. Between the colloid impermeable membranes of capillary and cell generally lies the protein-poor interstitial menstuum. So long as interstitial fluid separates cell membrane from capillary membrane the increased effectiveness of colloid osmotic pressure in the capillary following the hypotension of hemorrhage should be exerted only against the interstitial fluid, and the intracellular system should not be disturbed. However, wherever capillary wall lies in contiguity with cell wall, cell fluid also will come under the influence of the increased effective oncotic pressure in the capillaries, and loss of cell fluid into the capillary take place. Presumably a depletion of the interstitial reservoir would favor this mechanism of reduction in cell fluid volume.

The operation of still another factor must be considered in relation to flow of fluid from the tissues into the capillary bed after hemorrhage, and this is the force termed tissue tension, or tissue turgor. Such tissue tension is little understood and lends itself poorly to analysis. Presumably tissue tension is produced by such factors as the distending force of the intracapillary hydrostatic pressure exerted against the interstitial menstuum and cells, muscle tonus, and gravity. On the reduction in capillary filtration pressure, tissue tension conceivably should favor movement of fluid from the interstitial areas and cells toward the capillary bed. In the present state of our knowledge of the forces involved this can only be conjecture.

Possibly concerned also in the redistribution of

body fluids after hemorrhage is an alteration in the permeability of the capillary endothelium and cell wall incident to anoxemia and a reduced rate of removal of tissue catabolites. Such a disturbance in membrane function might relate itself not only to the relative impermeability for colloid but also to the selective impermeability of cell wall for specific ions.

Let us consider now the data shown in Table II and compare the concentration of electrolyte in the blood serum before hemorrhage and again twelve hours afterwards, but before water has been taken. The serum sodium falls distinctly, potassium falls, and chloride remains unchanged. Interstitial fluid contains a lower concentration of sodium than plasma (8, 9, 10), while intracellular fluid probably contains no sodium. Therefore, the inflow into the plasma of interstitial and intracellular fluid will necessarily reduce plasma sodium. On the other hand, interstitial fluid contains a higher concentration of chloride than does plasma while intracellular fluid contains no chloride. The effect on plasma chloride concentration, then, will depend upon the relative amounts of fluid brought into the circulation from the interstitial areas and from the cells, assuming alert renal function. One sees in Table III, that the concentration of serum sodium twenty-four hours after hemorrhage (water having been drunk) was invariably reduced, whereas the concentration of serum chloride was at times increased, at times decreased. In Table II serum potassium twelve hours after hemorrhage (no water drunk) is slightly reduced, in spite of the large amount of potassium carried from intracellular fluid into the urine. The urine excreted during this period had a potassium concentration of 344 m.eq. per liter, or about three times that of cell water. From this it is apparent that cell water was retained in the plasma while the cell electrolytes, unsuitable for plasma construction, were excreted.

In Figure 1 the data obtained in Experiment 36-23 and illustrative of the findings in the other experiments are shown. The power of the kidney to maintain conservation of the needful electrolytes sodium and chloride even after hemorrhage and a posthemorrhagic diuresis is well demonstrated.

A striking and invariable event in these experiments is illustrated in Figure 1. Without exception a diuresis to the extent of 20 to 500 per cent of the average urine volume before hemorrhage occurred during the second day after hemorrhage. This polyuria does not appear to be directly related to water intake. Its mechanism is obscure and will be the subject of further investigation.

#### SUMMARY

1. Measurements were made of the daily urinary excretion of sodium, potassium, chloride, and nitrogen by fasting dogs before and after hemorrhage to the extent of 2 to 3.5 per cent body weight.
2. Daily water intake and urine volume were measured.
3. Serum concentration values of the appropriate electrolytes and nitrogen were determined before and after hemorrhage.
4. A sharp increase in urinary potassium immediately after hemorrhage was noted, whereas sodium, chloride and total nitrogen in urine showed no significant change.
5. Regardless of whether water was drunk, hemorrhage was followed by a fall in serum sodium, potassium and protein, while chloride,  $\text{CO}_2$  and nonprotein nitrogen were variable.
6. A posthemorrhagic diuresis occurred invariably on the second day after hemorrhage.

#### CONCLUSIONS

During the process of compensating for blood loss fluid is drawn into the circulation from tissue cells as well as from interstitial areas. The potassium diffusing out of the cells in this process is promptly excreted in the urine.

The authors wish to acknowledge a debt of gratitude to Dr. James L. Gamble for stimulating their interest in the subject of body fluid metabolism and for helpful criticism in the preparation of this paper.

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# THE EFFECT OF DIETARY PROTEIN ON THE UREA CLEARANCE OF CHILDREN WITH NEPHROSIS<sup>1</sup>

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In so-called "pure lipid nephrosis" in children the urea clearance is frequently elevated markedly above the normal level. There also may be elevated urea clearances in some children with hemorrhagic Bright's disease with a decided nephrotic component. The nature of this paradox of renal hyperfunction in the presence of renal involvement is not entirely clear, and the present work was undertaken in an attempt to elucidate the rôle of the diet in this phenomenon.

The influence of the protein intake on the urea clearance has been studied in adults, but not in children. Cope (1) observed that in adult patients with nephritis, who had a normal or nearly normal clearance, there was a depression of the clearance when the protein intake was lowered from the moderate level of 75 grams per day to 40 grams. He found, however, that there was no increase in the clearance when the protein intake was raised from 75 to 125 grams. In adult patients with chronic renal disease sufficiently advanced to depress the urea clearance consistently below 50 per cent of normal, the effect of lowering the dietary protein was relatively slight. In such patients the urea clearance on 40 grams of protein per day remained about 90 per cent of the clearance on 75 grams. In 1934, Goldring, Razinsky, Greenblatt and Cohen (2) extended Cope's observations to normal men, and came to the same conclusions regarding normal men that Cope arrived at in regard to the patients with unimpaired ability to excrete urea. In Goldring's observations the diet was varied within somewhat greater limits. On the low diet there was observed an average reduction of the clearance of 23 per cent. High protein intakes caused no elevation above the usual normal clearance, even when the protein intake was pushed up to 280 grams per day.

In the present study we have extended to ne-

phrotic children the study of the relation between protein intake and clearance level.

## CONDITIONS OF OBSERVATION

During the experimental period no attempt was made to keep the fat and carbohydrate intakes constant, as previous observations by Page and Farr (3) on the influence of high and low fat diets on plasma lipids had shown these food factors, as well as the total caloric intake, to be without significant influence on the clearance. The salt intake was kept constant throughout the period of observation. The caloric intake was kept relatively constant. The diets were prepared under the supervision of Miss G. Drew and the success in keeping the children eating satisfactorily and contentedly on the various diets is due in no small measure to her efforts.

Four patients with nephrosis, all four years of age, were observed for a period of 54 days on diets with varying protein contents. All of the patients had been observed for several months prior to this study and the maximum variations in the urea clearance, in the absence of complicating intercurrent disease factors, were known. In each patient selected for study, the urea clearance was consistently elevated above normal. Figures 1, 2, 3 and 4 show the clinical, laboratory and diet details relating to these patients since admission.

The changes in clearance reported in this paper were observed over seven successive periods, as indicated in Table I.

To each period several days were allowed for the dietary change to produce its effect on renal function; then urea clearances were observed on two successive days.

Before the second clearance in Period 4 was done, the patient was given enough urea by mouth to bring the blood urea nitrogen up to about the level noted on the high protein diet in the first period, and one hour clearances were run, with blood urea determinations done each hour. The object

<sup>1</sup> Read in abstract before the Society for Pediatric Research May 5, 1936.

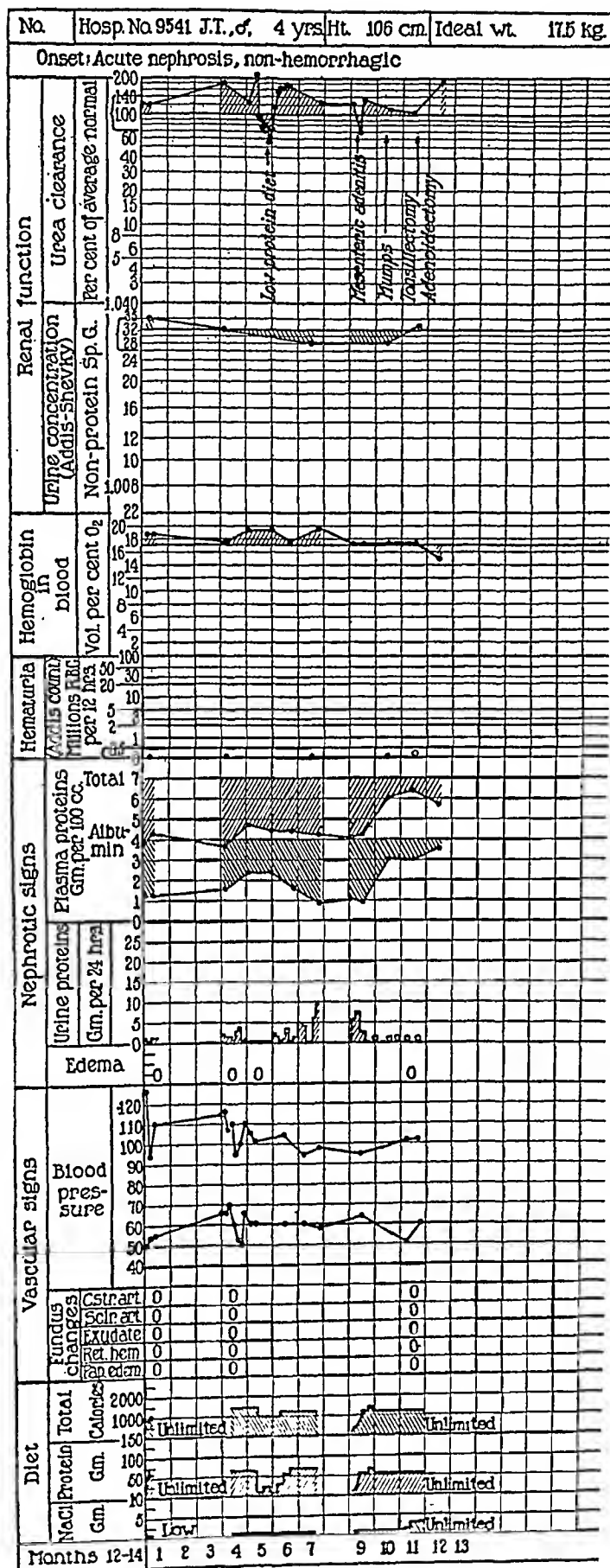


FIG. 1.

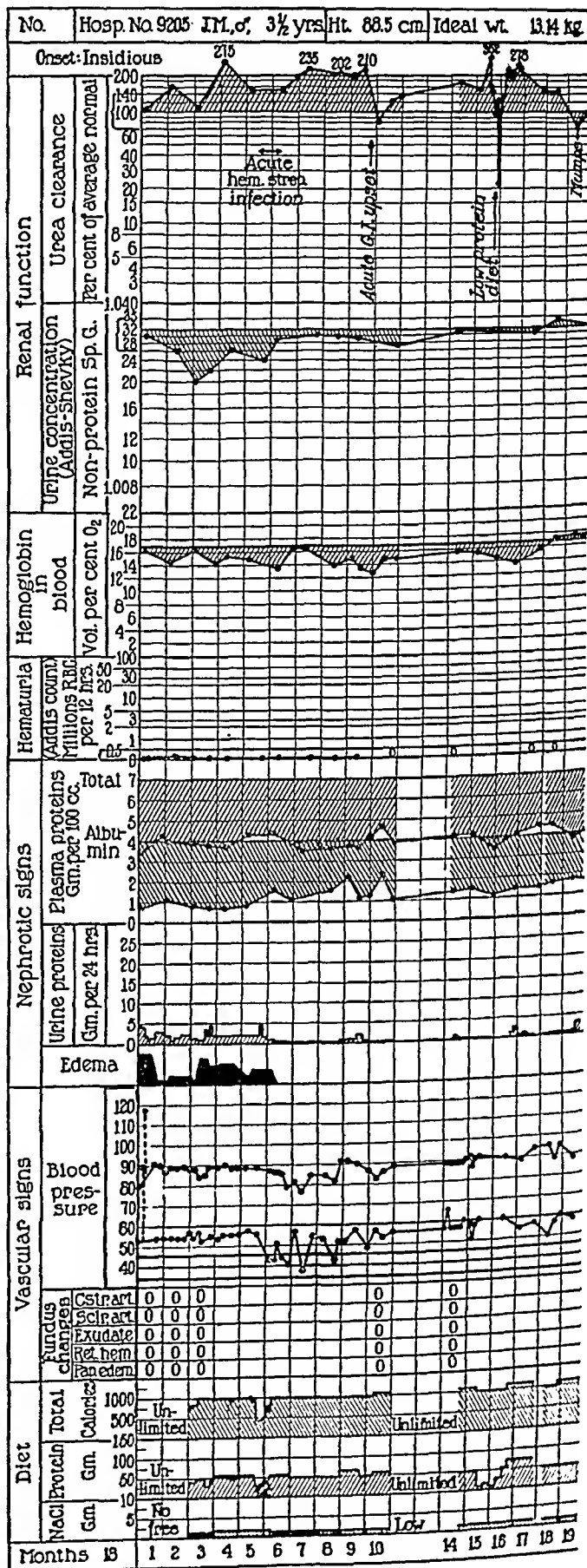


FIG. 2.

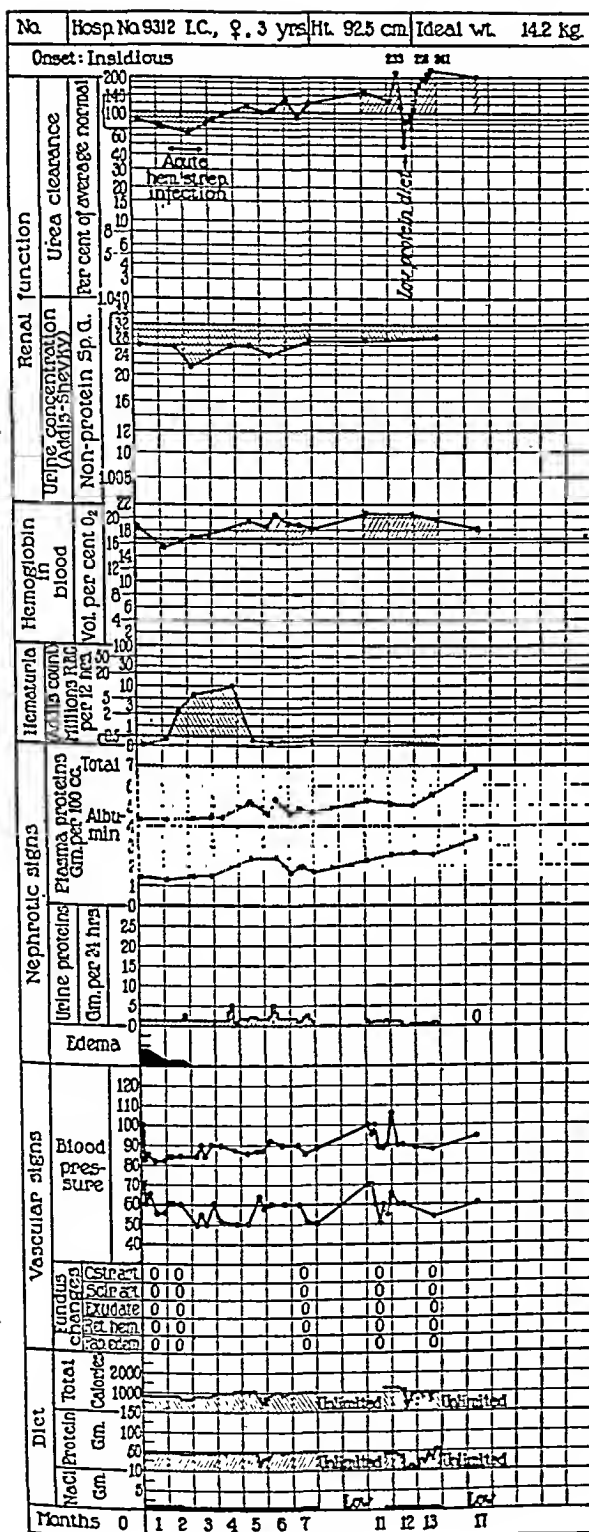


FIG. 3.

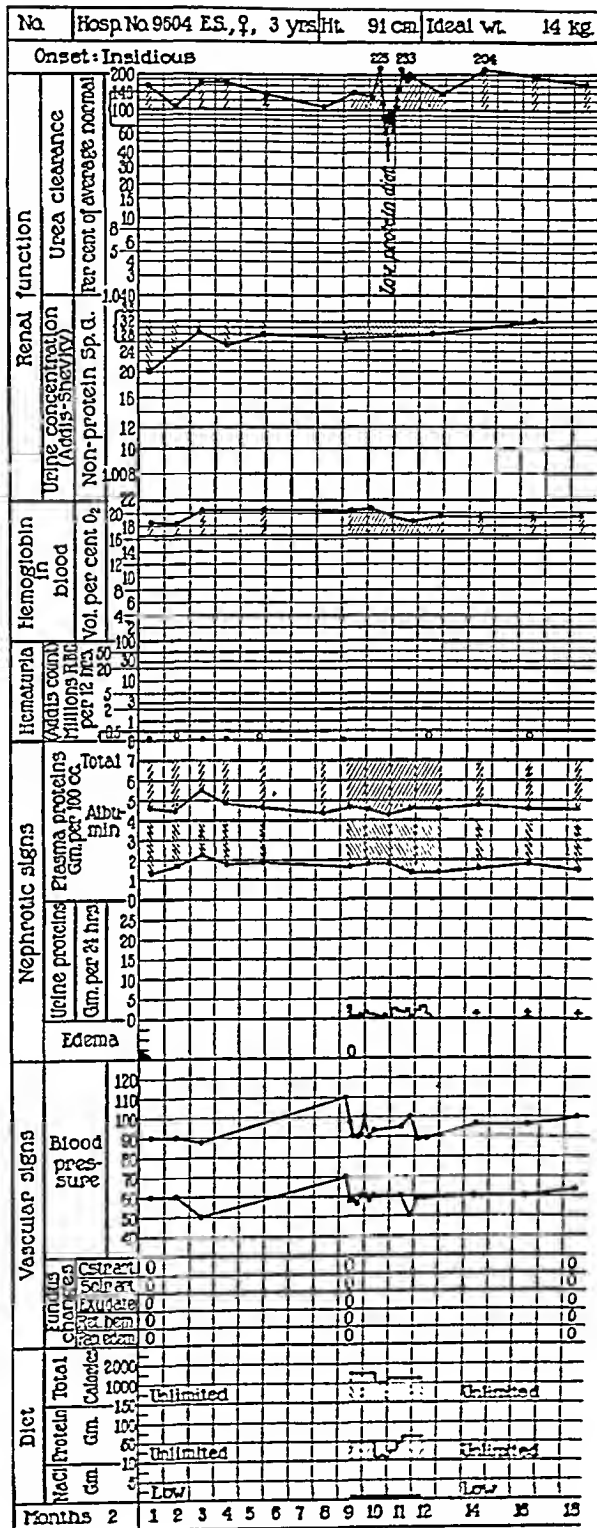


FIG. 4.

FIGS. 1, 2, 3, AND 4. CLINICAL COURSE OF PATIENT SHOWING VARIATIONS OF UREA CLEARANCE AND OTHER CLINICAL OBSERVATIONS PRIOR TO STUDIES SHOWING THE EFFECT OF PROTEIN IN DIET.



TABLE I  
*Duration of individual dietary periods and approximate protein intake during each period*

Period number	Duration of period	Approximate protein intake per kilo body weight
	<i>weeks</i>	<i>grams per day</i>
1	2	3.0
2	1	0.5
3	1	1.0
4	1	0.5
5	1	2.0
6	1	3.0
7	1	4.0

of feeding urea was to ascertain whether the increase of clearance caused by high protein feeding could be duplicated by merely raising the blood urea to the previously existing level. At the same time creatinine clearances were done.

The children were well throughout the entire period of observation, and with one exception (one patient had an acute gastric upset on the second low protein diet lasting one day) took the diet satisfactorily for the entire interval. Throughout this study, with the exception previously noted in Period 4, the clearances were run over two consecutive 12-hour periods instead of the more usual one-hour periods. During part of the studies one-hour clearances were done simultaneously in the usual manner, and these showed changes in the same direction, but the swings were not so wide as in the 12-hour tests. The longer intervals appeared to reflect more accurately the variations in renal function related to the diet. Then, too, over long intervals the collection of urine in children is much more exact. The 12-hour clearances are on the whole somewhat higher on the high protein diet and somewhat lower on the low protein diet than were the one-hour clearances.

#### ANALYSIS AND CALCULATION

The analyses of blood and urine urea were done by the hypobromite technique of Van Slyke and Kugel (4). The ammonia nitrogen was not removed from the urine. Consequently, the clearances calculated are approximately those of urea + ammonia. Van Slyke, Page, Hiller and Kirk (5) found that in human subjects clearances of urea + ammonia were more consistent than sim-

ple urea clearances, when the ammonia excretion was increased to important proportions.

The urea clearance was calculated by the formula of Möller, McIntosh and Van Slyke (6), with the measured urine volume corrected for surface area according to McIntosh, Möller and Van Slyke (7). The urinary protein was determined by the rapid sedimentation method of Shevky and Stafford (8). Urinary chlorides were estimated by the modified Volhard-Harvey titration as described by Peters and Van Slyke (9). For the first creatinine clearances which were done before this period of observation was commenced but when the patients were on comparable protein diets, the creatinine was determined in the urine colorimetrically by Folin's method (10) and in the blood by the method of Folin and Wu (11). The clearances were calculated as advocated by Rehberg (12). The creatinine clearances done on the low protein diet in this study were carried out according to the method described by Hanzal and Hayman (13). In all instances the normal creatinine clearance was taken as 148 cc. for an average adult and the urine flows were corrected for body size in the same fashion as noted above in calculating the urea clearances.

#### RESULTS

The results correlated with the diet are shown in Figure 5 and Table II. It will be noted that there is a precipitous fall in the urea clearance when the protein is cut from 3 grams per kilo to 0.5 gram. This fall begins immediately although the minimum level is not reached for about three days. When, subsequently, one gram of protein per kilogram was fed, the clearance was somewhat higher, but when 2 to 2½ grams per kilogram were fed the urea clearance rose further to near its maximum value. Further increases in protein intake to 3 and 4 grams per kilo affected the urea clearance according to the law of diminishing returns.

The magnitude of these changes is perhaps better brought out by a consideration of the composite curve obtained by averaging the results on all of the children (Figure 6). The extraordinarily high clearances observed on the high protein diets indicate an effect on renal function of altogether greater degree than was observed in

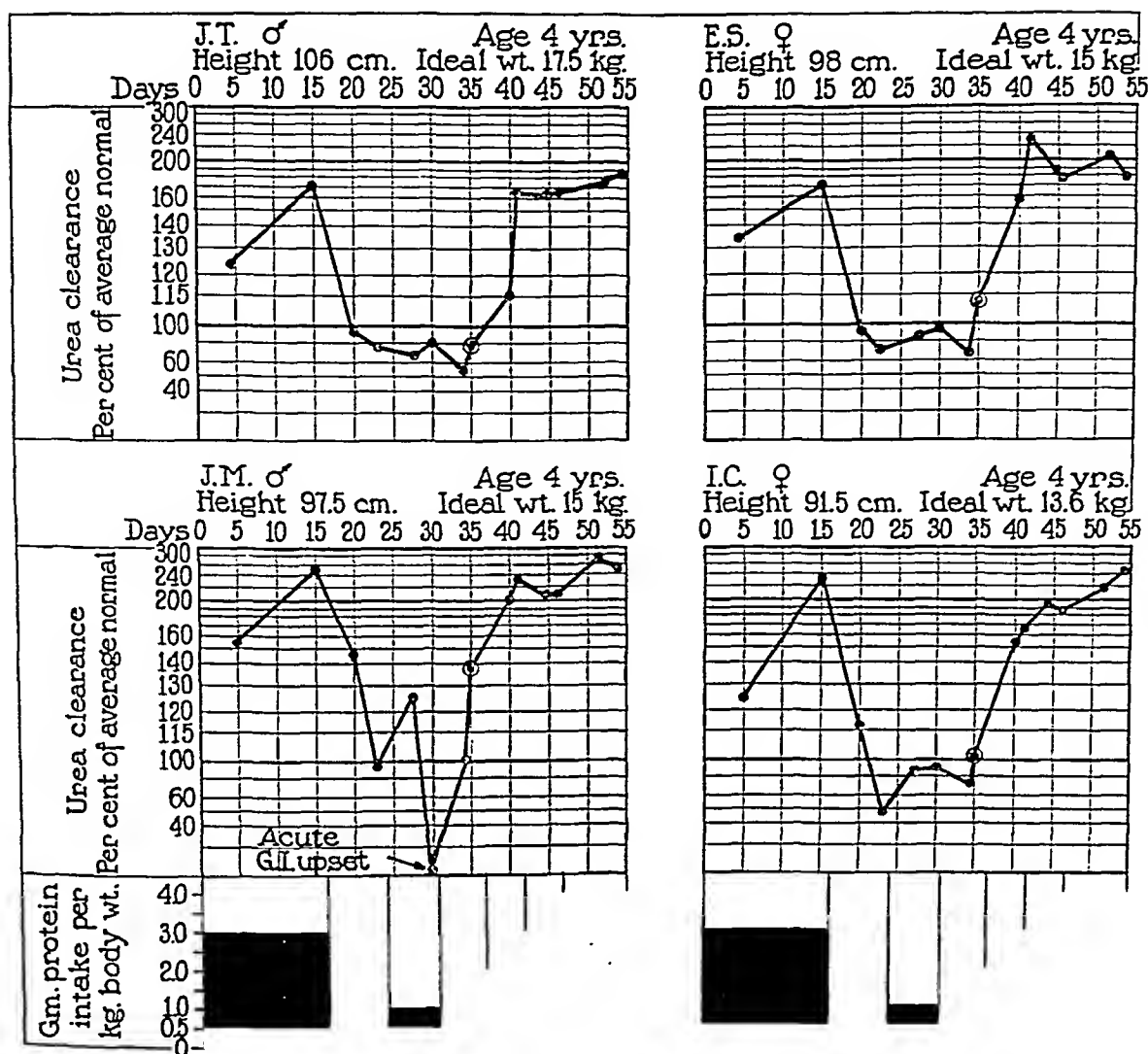


FIG. 5. RELATION OF 12-HOUR UREA CLEARANCE TO VARIATIONS IN PROTEIN INTAKE IN THE FOUR OBSERVED PATIENTS.

The encircled point is a one-hour clearance at which time urea and creatinine were fed.

adults, nephritic or normal, by either Cope (1) or Goldring *et al.* (2).

When sufficient urea was given by mouth to increase the blood urea to a value somewhat higher than that found when the protein intake was at a maximum, the average effect on the clearance was only an increase from 73 per cent normal to 103 per cent normal. This variation is within ordinary normal limits, and the data are not sufficiently numerous enough to show whether it has any statistical significance. Compared with the great clearance changes caused by protein feed-

ing, the changes caused by urea feeding are insignificant, as has been found in previous reports from this clinic (Møller, McIntosh and Van Slyke (6); Van Slyke, Alving and Rose (14)). It appears that the protein effect on the clearance is chiefly due to metabolites other than urea.

The ratio of per cent normal function as measured by the creatinine clearance to the urea clearance (per cent normal function by creatinine clearance/per cent normal function by urea clearance) was 1.46 on the low diet. Creatinine clearances on these patients done previously when an

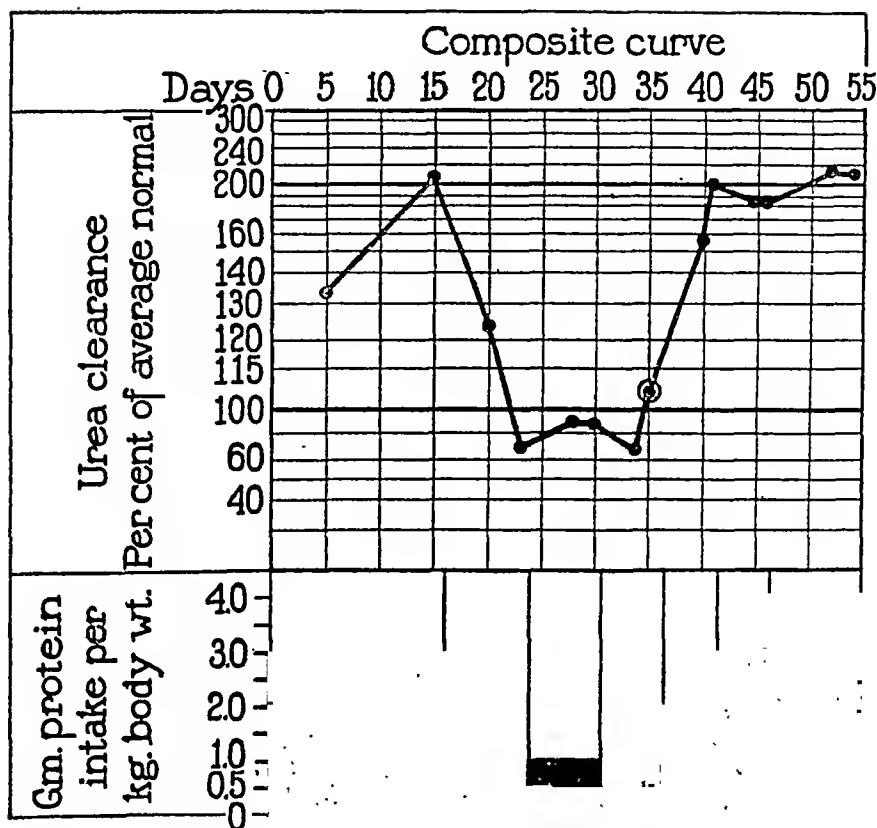


FIG. 6. COMPOSITE UREA CLEARANCE CHART SHOWING AVERAGE CHANGE IN THE 12-HOUR UREA CLEARANCE WITH CHANGE IN PROTEIN INTAKE.

The encircled point is an average of the one-hour urea clearances, when urea and creatinine were fed.

optimum diet of 3 grams of protein per kilo was being fed showed a ratio of 1.69. The differences in these two ratios are probably not significant; the change in the creatinine clearance was of the same magnitude as the urea clearance.

Since the clearance changes are reversible it is unlikely that the low clearance on the minimum protein diet represents any loss of excretory ability. Van Slyke, Rhoads, Hiller and Alving (15) have shown that in dogs the urea clearance can, as in our patients, be caused to vary over a wide range by shifting the protein intake, and under these circumstances the clearance varied parallel with the renal blood flow. It appears probable that the clearance changes observed in our patients may be similarly due to variations in renal blood flow, caused by the influence of protein metabolites, chiefly other than urea.

The effects of protein intake on urea clearance differ markedly in our 4-year old patients from the effects noted in adults by Cope (1) and by Goldring *et al.* (2). The chief difference is that

in the adults observed by these authors, increase of protein intake to levels markedly above the usual did not cause increase of the urea clearance. Our results with children are much more like those obtained with dogs by Jolliffe and Smith (16) and by Van Slyke, Rhoads, Hiller and Alving (15). Under the influence of maximal variations in protein intake the latter authors obtained in dogs' blood urea clearances varying from 15 to 90 cc. of blood per square meter of body surface. Urea as the stimulating factor was excluded in these experiments by feeding urea enough to cause the same rise in its blood concentration noted in the high protein periods; the urea caused no significant increase in renal function or blood flow.

Because of lack of available normal subjects, our observations on children with the nephrotic syndrome lack control observations on healthy children. It therefore remains for future work to ascertain whether the lability of the renal function under varying protein intake observed in our

patients was due to the nephrotic condition, or whether it is common to normal children of the same age.

One important aspect of this response of the kidney to protein intake is that one must be cautious in interpreting high urea clearances in children unless the dietary history for the preceding few days is known. The difficulties affect only high clearances, as the clearance apparently cannot be made to drop by low protein diets so far as to suggest severe renal damage in its absence.

The secretion of urinary chloride (Table II) fell in these patients at the time the protein intake was diminished. Since all of these patients excrete chloride only with difficulty and in addition were on chloride restricted diets, the actual reduction in chloride excretion was not large but the direction of change was the same in all of the patients. This may in part be the effect of a high

protein diet in assisting in controlling the edema in patients of this type.

Albuminuria decreased rather sharply in these subjects when they were placed on the low protein diets (Table II). This occurred almost immediately after the inception of the low diet. With return to the higher protein intake the albumin excretion came back to its original levels. If one accepts the hypothesis that the albumin in the urine in patients of this type comes largely from the plasma as glomerular filtrate, then any drop in protein excretion such as was seen in this series of observations may be occasioned by decreased glomerular filtrate, and is not necessarily the result of any decrease in permeability of the glomerular tufts to protein. Under these circumstances the daily output of urinary protein would be proportional to the daily volume of glomerular filtrate and not to urine volume. Bing (17) has recently reviewed the literature on proteinuria and has also presented original evidence which supports the belief that protein in the urine is derived almost exclusively from glomerular filtrate, and in a given subject varies proportionally to the glomerular filtrate.

TABLE II

*The relation between protein intake, proteinuria, urinary chloride excretion and average urea clearance during period of observation. The chloride intake approximated 1.0 gram per day for each patient*

Patient	Approximate protein intake	Duration of diet	Chloride in urine	Protein in urine	Average urea clearance	Number of observations of proteinuria and urinary chloride
	grams per kilo	days	grams per day	grams per day	per cent normal	
I. C.	3.0	16	0.70	1.62	179	6
	0.5	8	0.06	0.09	82	2
	1.0	6	0.17	0.36	86	2
	0.5	5	0.12	0.51	74*	2
	2.0	6	0.61	0.80	164	2
	3.0	6	0.26	0.84	192	1
	4.0	7	0.44	0.95	230	4
J. M.	3.0	16	0.40	0.44	205	6
	0.5	8	0.10	0.20	120	2
	1.0	6	0.10	0.09	127†	2
	0.5	5	0.01	0.20	100*	2
	2.0	6	2.1	0.16	217	2
	3.0	6	0.95	0.34	208	1
	4.0	7	0.44	1.07	258	4
E. S.	3.0	16	0.51	2.47	151	6
	0.5	8	0.10	0.53	80	2
	1.0	6	0.10	0.92	87	2
	0.5	5	0.45	0.55	65*	2
	2.0	6	0.85	2.7	195	2
	3.0	6	0.76	2.0	180	1
	4.0	7	0.41	2.01	190	4
J. T.	3.0	16	0.88	0.60	148	6
	0.5	8	0.35	0.06	87	2
	1.0	6	0.42	0.41	73	2
	0.5	5	0.03	0.35	54*	2
	2.0	6	0.15	2.05	142	2
	3.0	6	1.08	0.7	162	1
	4.0	7	0.92	1.72	178	4

\* Does not include 1-hour clearances when urea was fed.

† Clearance taken during acute gastro-intestinal upset not included for average.

## CONCLUSIONS

In 4 children, aged 4 years, with the nephrotic syndrome, the urea clearance was found to vary markedly with the protein intake. Protein intakes of 0.5, 1, 2, 3, and 4 grams per kilo per day were accompanied by average urea clearances of 73, 88, 178, 184, and 216 per cent of mean normal, respectively.

The creatinine clearance showed variations similar to those of the urea clearance.

Administration, during low protein periods, of urea sufficient to produce urea outputs like those observed during high protein intake caused relatively slight increase in urea clearance. It appears therefore that the stimulus of renal function by high protein diets was due to products other than urea.

The effect of varying protein intake on urea clearance in our patients was similar to the effect observed in dogs by Van Slyke, Rhoads, Hiller and Alving (15). It therefore appears probable that, as demonstrated in their experiments, the clearance changes indicated parallel changes in renal blood flow.

The effects of protein intake on urea clearance in our 4-year old patients were much greater in magnitude than similar effects observed in adult human subjects, either normal or with renal disease.

It remains to be ascertained by control observations on normal children whether the observed lability of the clearances is common to young children, or in our patients was due to the combined effect of early age and the nephrotic syndrome.

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# THERAPEUTIC SERUM FOR PNEUMOCOCCUS TYPE V (COOPER) PNEUMONIA<sup>1</sup>

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The characteristics of the pneumonias caused by pneumococcus Type V (Cooper) will be described elsewhere. This type was originally designated Type IIA by Avery. It is frequently mistaken for Type II, but by means of the Neufeld technic it is readily distinguished from that type. In general, pneumococcus Type V causes a less severe pneumonia than pneumococcus Type II. In the last seven years 249 cases of pneumonia due to Type V were observed at Harlem Hospital among adults. There were 34 deaths among 163 cases in which serum was not used, and the mortality rate was 20.8 per cent. If we add to these the 6 cases who came in with an overwhelming blood invasion and who died before they received a dose of serum adequate to affect the outcome, the mortality would be even higher. The 163 patients, who were not treated specifically and the other 6 cases who entered the hospital with overwhelming bacteremia constitute 169 cases; among them there were 40 deaths, a mortality of 24.4 per cent.

## *Therapeutic serum. Selection of cases*

Pneumococcus Type V was responsible for seven and one-half per cent of the pneumococcus pneumonias observed in adults at Harlem Hospital during the past seven years. During the year 1934-1935, the incidence of this type was 10.8 per cent among 521 cases of pneumococcus pneumonias. Because of the frequency of pneumonias caused by pneumococcus Type V, and their considerable mortality, it, among other types, was selected for treatment with specific serum.<sup>2</sup>

<sup>1</sup> This study received financial support in part from the Metropolitan Life Insurance Company and from the Altman Foundation, Inc.

<sup>2</sup> The horse serum was prepared by the Department of Health at the Municipal Farm, Otisville, New York, with funds provided in part by the City of New York; in part

When serum was available, the cases were alternated for serum treatment. At first, the serum was administered every eight hours. When our experience with this and other types showed the advantage of more intensive treatment, larger doses were administered, and the time between doses was shortened to three, or even two hours. Eighty-six patients received serum in the seven years covered by these observations, and 163 served as controls and received no serum. Nineteen of the patients who received serum have been excluded from the statistical evaluation because of inadequate treatment. Of these, 11 died and 8 recovered. The cases were removed from the series as inadequately treated if they were bacteremic and received less than 200,000 units within 24 hours, and if they were non-bacteremic and had received less than 100,000 units within 24 hours. They were not removed from the series even though less than these amounts of serum had been given, if sufficient had been administered to show agglutinins for Type V pneumococcus in the blood. Among the recovered cases, 4 were excluded because the temperature was already descending at the time the serum was given, and it was felt that the serum had had no demonstrable influence on the disease.

## *Effect on death rate*

There were 67 cases adequately treated with serum. Of these, 5 died, a mortality of  $7.5 \pm 3.2$  per cent. There were 163 cases treated without serum, of whom 34 died, a mortality of  $20.8 \pm 3.2$

by the Altman Foundation, Inc.; and, by the Littauer Pneumonia Research Fund. It was also prepared by the Lederle Laboratories at Pearl River, New York. It was refined at the Department of Health Laboratories at first by Banzhaf and later by Falk. At Pearl River it was refined by Joseph Greene. At first the serum contained only 500 units per cc. Later serum containing as much as 8000 units was produced.

TABLE I  
*Pneumococcus Type V (Cooper) pneumonia*

Years	Patients adequately treated with serum			Patients not treated with serum			Average units of antibody in serum
	Number of cases	Deaths		Number of cases	Deaths		
		Number	Per cent		Number	Per cent	
1928 to 1933	26 11*	3 2*	11.5 18.1*	108 28*	23 15*	21.3 53.5*	782
1933 to January 1936 †	41 6*	2 2*	4.9 33.3*	55 15*	11 11*	20.0 73.3*	3163

\* Indicates bacteremia.

† During six months from January 1st to June 30th, 1936, there were 25 cases treated with serum with one death (bacteremia) or 4 per cent and 10 cases not treated with serum with one death (bacteremia) or 10 per cent.

per cent. The ratio of the difference to its error in this instance is 2.7, which indicates that the chances are 993 in a thousand that the difference in mortality rate was due to the difference in the treatment and not fortuitous.

Seventeen of the cases treated with serum were bacteremic; 4 died, a mortality rate of  $23.5 \pm 10.3$  per cent. Among 43 bacteremic cases not treated with serum there were 26 deaths, a mortality rate of  $60.5 \pm 7.4$  per cent. The ratio of the difference to its error in this instance is 2.9. Of the 50 cases treated with serum and not bacteremic, only one died, a mortality rate of 2.0 per cent. Of the 120 non-bacteremic cases cared for without serum, 8 died, a mortality rate of  $6.7 \pm 2.3$  per cent. The ratio of the difference to its error is 1.5.

If we divide our experience into that before and that after 1933 as shown in Table I, we find that before 1933 the mortality in cases treated with serum was approximately one-half of that in those not treated with serum. In the period before 1933 the average potency of serum was 782 units per cc. In the later period, the average potency was 3162 per cc. The death rate in the later period was still further reduced and became approximately one quarter of that in the cases not treated with serum. The death rate in the cases untreated with serum was approximately the same in both periods: 21.3 per cent in the first period and 20 per cent in the second period. With the increase in the strength of serum, the number of units given to the non-bacteremic patients was increased from an average of 152,000 units of 500-unit average potency per cubic centi-

meter in 1929-1930 to an average of 383,500 units of 6,649-unit average potency per cubic centimeter in 1935-1936. Larger amounts were given to bacteremic patients.

#### *Influence of serum on bacteremia.*

Among the 67 cases adequately treated with serum, 17 were bacteremic, an incidence of 25.1 per cent. In the 163 cases not receiving serum therapy, 43 had bacteremia, an incidence of 26.4 per cent. After serum treatment was commenced, there was no invasion of the blood stream in any cases treated adequately.

It was possible to determine the day on which the blood was invaded in 22 cases. This occurred before the 4th day in 4 cases, and after the 4th day in 18 cases. Of the fatalities among cases treated with serum the blood was sterile in only one, and in this case treatment was begun on the 6th day.

Partition of the cases with respect to degree of invasion revealed that there were fewer cases with high colony counts in those who had adequate treatment with serum. The mortality rate was as low as 14.3 per cent if sufficient serum was given and the number of colonies in the blood was less than 50 per cc. The largest number of colonies in any culture was taken to designate the group to which a case belonged. It was found that the use of serum prevented increase in the number of colonies. From these data it seems fair to conclude that with adequate dosage of specific serum, not only is the blood protected against invasion, but also that the colonies do not increase after the serum is administered should the blood have been invaded already.

### Duration of disease

Among the 67 patients who received serum, in 40, or 59.7 per cent, the fever terminated by crisis on the same day or the day following treatment with serum. Among the 163 cases not treated with serum such prompt defervescence after hospitalization occurred in only 13 instances, or 8 per cent. The termination of the disease seemed to be directly related to the administration of the specific serum.

There was one case, treated very late in the disease, where death may have been hastened through the administration of serum. This patient, a man aged 45, had bacteremia, was cyanotic, dyspneic and distended. His pulse was 120, and his respirations were 48. The patient received a dose of 5 cc. and a dose of 8 cc. serum without reaction on the 12th day. Two hours later the pulse was weak and distention had increased. After the third dose of serum, 5 cc. (7500 units), was injected he felt hot, retched, passed gas and feces, and was in collapse. Adrenalin was given without avail.

The following case exemplifies the value of specific serum when properly used in the treatment of a patient suffering from pneumonia due to pneumococcus Type V:

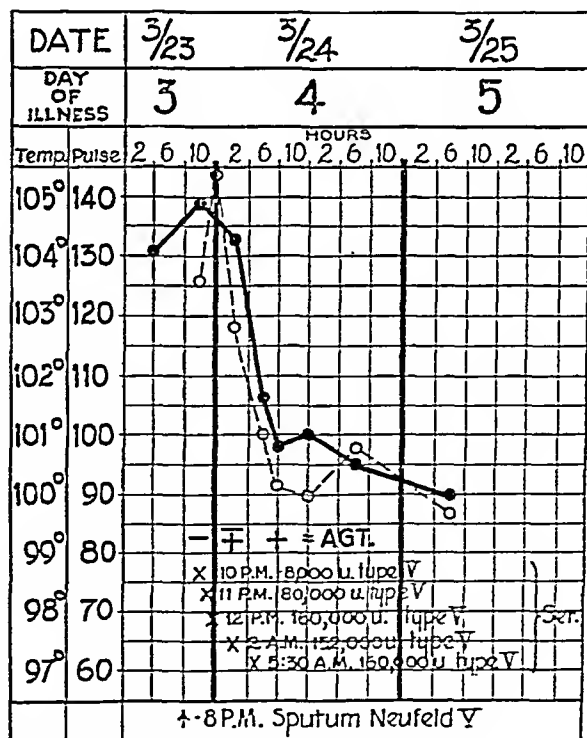
*Case A. G.*, a plasterer, age 67, had a history of chronic bronchitis and emphysema lasting 40 years, with recurrent attacks of paroxysmal ventricular tachycardia. He was taken ill at 8 a.m. on March 21, 1936 with nausea, precordial pain, fever, and headache. The pain was not aggravated by cough or by deep inspiration. On March 23 the cough became worse, and was followed by the expectoration of white tenacious sputum which was bloody on one occasion. He had no chills and no vomiting. On March 23, at 9 p.m. he complained of severe headache and mild substernal pain. There was no pleuritic pain. The temperature was 104.6° F.

*Physical examination.* An acutely ill, aged man, dyspneic, tachypneic, apathetic, and slightly delirious. His tongue was dry and coated, the tissue turgor was poor. *Chest.* Dulness, bronchial breathing, and bronchophony was heard over the left lower chest posteriorly. Slight dulness, bronchovesicular breathing and a moderate number of crepitant râles were present over the right lower chest posteriorly. Respirations were 44. Heart was normal in size, regular sinus rhythm with numerous ventricular premature contractions. Ventricular rate was 132, and pulse rate 126. There was cyanosis of the lips and nail beds. Abdomen was not distended.

Conjunctival and dermal tests with horse serum were

negative. At 10 p.m. he received 8,000 units (1 cc.) of Type V serum. At 11 p.m. he received 80,000 units (10 cc.); at midnight, 160,000 units; at 2 a.m. 152,000 units; and at 5:30 a.m., 160,000 units. At 6 a.m. the temperature had fallen to 100.4° F. and the pulse to 92. From that time on the pulse and temperature were normal. All premature contractions disappeared. Before serum was commenced, there were no agglutinins in the blood of the patient. Before the 4th injection agglutinins in the blood were  $\pm$  and at 8 a.m., after 56,000 units of serum had been administered the agglutinins were intensely positive. At this time he was clear mentally, had slight dyspnea, and requested food. The following day he was comfortable and requested a full diet; his dyspnea was gone.

This patient received the following during the night of treatment; chloral hydrate, grains 15; sodium bromide, grains 30; codeine sulphate,  $\frac{1}{2}$  grain. An oxygen tent was employed from 3 a.m. on March 24 until noon on March 25. On March 27 and on March 28 he received 1,000 cc. of 5 per cent glucose in saline intravenously. He was out of bed in a chair on March 31, ten days after the



Temperature = ● — — — — ● Pulse = ○ — — — — ○

FIG. 1. EFFECT OF SPECIFIC SERUM ON PULSE, TEMPERATURE AND AGGLUTININS IN CASE A. G., PNEUMOCOCCUS TYPE V PNEUMONIA.



onset of the illness and seven days after the commencement of serum therapy. One week later he was at his regular work as a plasterer.

#### CONCLUSION

It seems reasonable to conclude that the available specific Type V serum not only promptly terminates the illness, but also protects and clears the blood stream of these pneumococci. The death rate in the cases observed was greatly re-

duced. In a later series treated with more potent preparations of serum more frequently administered, the mortality rate was reduced to one quarter of that in the contemporaneous controls. This mortality rate cut in half the mortality rate of the earlier serum series. The mortality rate in the first series was half that of the controls. The mortality rate in the controls of both series was the same. In the combined series there were sufficient cases for statistically valid conclusions.

# OBSERVATIONS ON THE DEVELOPMENT OF THE HIGH BLOOD SEDIMENTATION RATE IN RHEUMATIC CARDITIS<sup>1</sup>

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The practical value of serial determinations of the erythrocyte sedimentation rate as an aid in detecting the presence of rheumatic activity has proved itself thoroughly among clinicians. Our findings during the last five years have been in complete accord with those of a number of other investigators (1 to 11, inclusive). The erythrocyte sedimentation rate is regularly elevated in patients with rheumatic carditis, and even minor fluctuations seem to be directly related to the clinical course of the disease. Except when associated with congestive heart failure, a decreasing sedimentation rate nearly always reflects diminishing activity of the rheumatic process. The present paper deals with the initial development of a rise in sedimentation rate at the onset of the rheumatic attack, and with an investigation of the factors in blood directly responsible for the change.

## *The erythrocyte sedimentation rate in pharyngitis, scarlet fever and rheumatism*

As shown in a previous publication (12), an attack of acute rheumatism is usually preceded by an upper respiratory infection with hemolytic streptococcus, which we designate as Phase I of

the rheumatic attack. Following recovery from the primary infection, there is a symptom-free interval of from one to three (rarely five) weeks in length, during which the patient appears to be in perfect health. This we designate as Phase II. The rheumatic attack proper is referred to as Phase III. It has been possible in a number of known rheumatic subjects who have been under close observation for a period of years to follow the sedimentation rate from the onset of hemolytic streptococcus pharyngitis through Phase II into Phase III or complete recovery as the case might be. Many of these patients developed typical acute rheumatism. Some escaped all signs of rheumatic recrudescence. The sedimentation curves were quite different, depending on whether the patient developed a rheumatic attack or not. Sample curves of both types are presented in Figures 1 and 2.

It will be seen from these curves that the sedimentation rate was slightly or moderately elevated during pharyngitis in some of the individuals studied, and remained at a normal level in the others, irrespective of whether the infection was followed by rheumatism or not. In patients who escaped recrudescences (Figure 1) the sedimentation rate remained at its normal level or returned to normal in about two to three weeks

<sup>1</sup> The work reported in this communication was carried out under The W. K. Kellogg Foundation Fund.

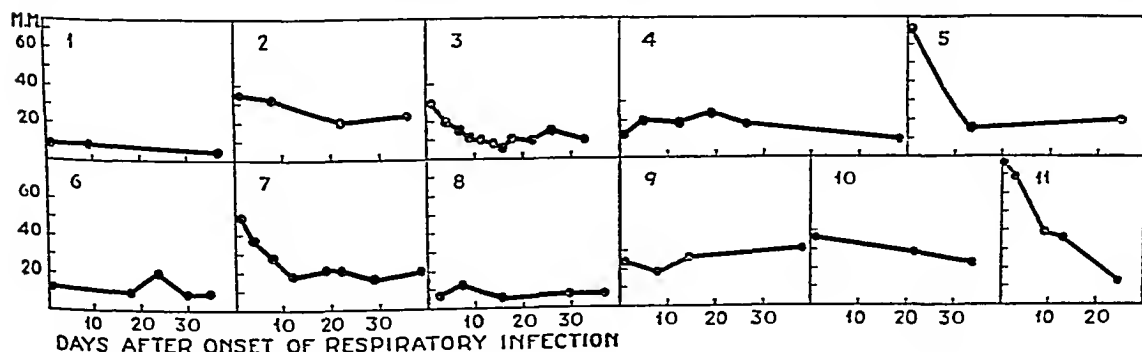


FIG. 1. SEDIMENTATION RATES OF RHEUMATIC SUBJECTS WITH HEMOLYTIC STREPTOCOCCUS PHARYNGITIS WHICH WAS NOT FOLLOWED BY RHEUMATIC SYMPTOMS.

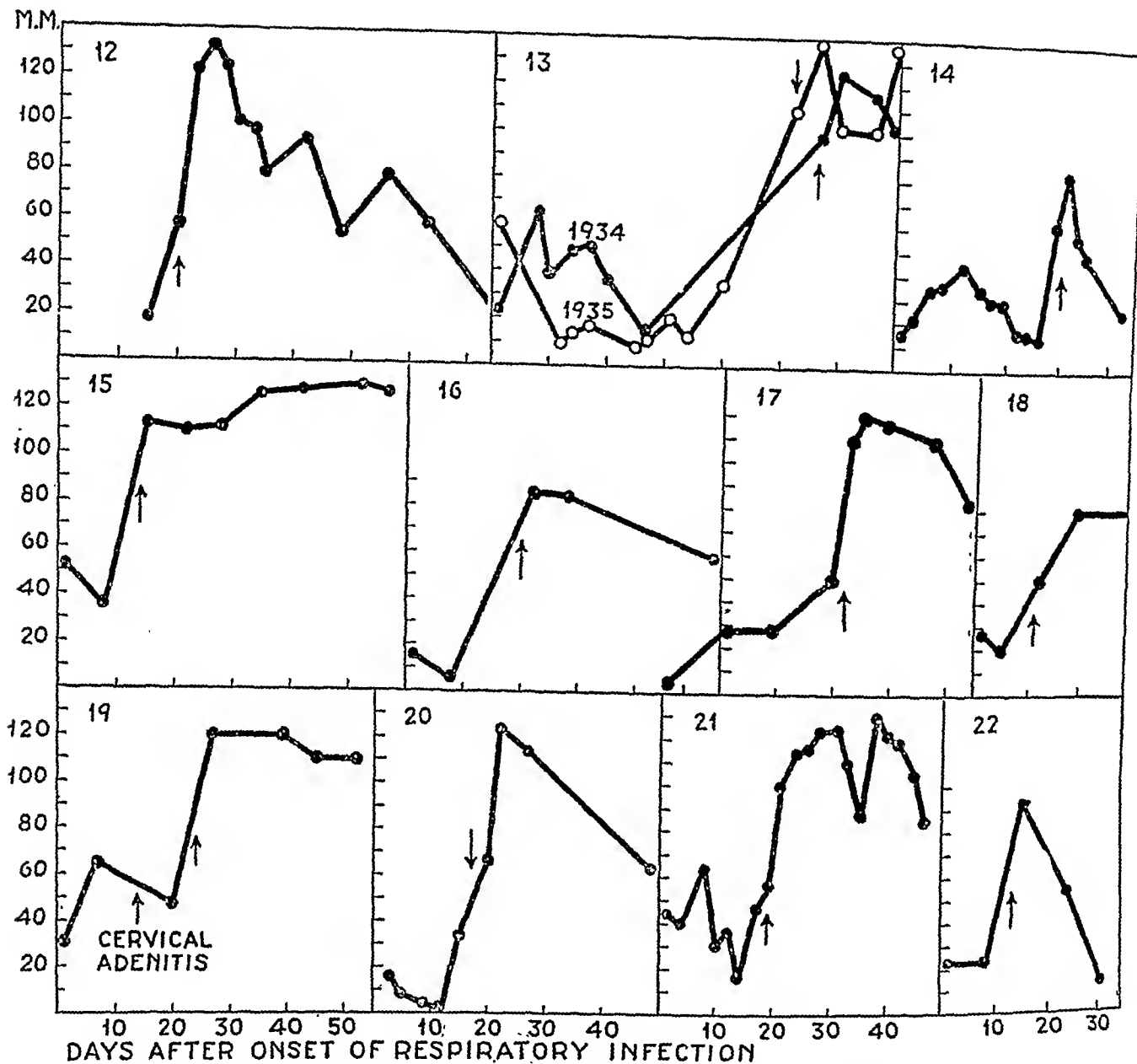


FIG. 2. SEDIMENTATION RATES OF RHEUMATIC SUBJECTS WITH HEMOLYTIC STREPTOCOCCUS PHARYNGITIS WHICH WAS FOLLOWED BY SEVERE RHEUMATIC CARDITIS (ONSET OF RHEUMATIC ATTACK INDICATED BY ARROWS).

from the onset of infection. In patients who developed rheumatic attacks, however (Figure 2), the subsidence of sedimentation rate after recovery from pharyngitis was interrupted by a second sharp increase coincident with or just preceding the onset of rheumatic symptoms.

A similar study was made of a control group of subjects with hemolytic streptococcus pharyngeal infections who had no history of previous rheumatic disease. This group consisted of (a) 15 young adults, mostly nurses, with acute pharyngitis, whose throat flora contained hemolytic streptococcus in predominance, and (b) 10 patients, mostly children, admitted to Willard

Parker Hospital with typical scarlet fever. The sedimentation rate curves are presented in Figure 3.

Eighteen of these patients recovered without developing complications. Their sedimentation rate curves are indistinguishable from those shown in Figure 1. Five patients with pharyngitis and two with scarlet fever did develop complications during convalescence. All of these complications were accompanied by renewed increases in the sedimentation rate. These secondary increases were moderate, with the exception of one patient who developed, successively, otitis, mastoiditis, mild polyarthritides and electrocardio-

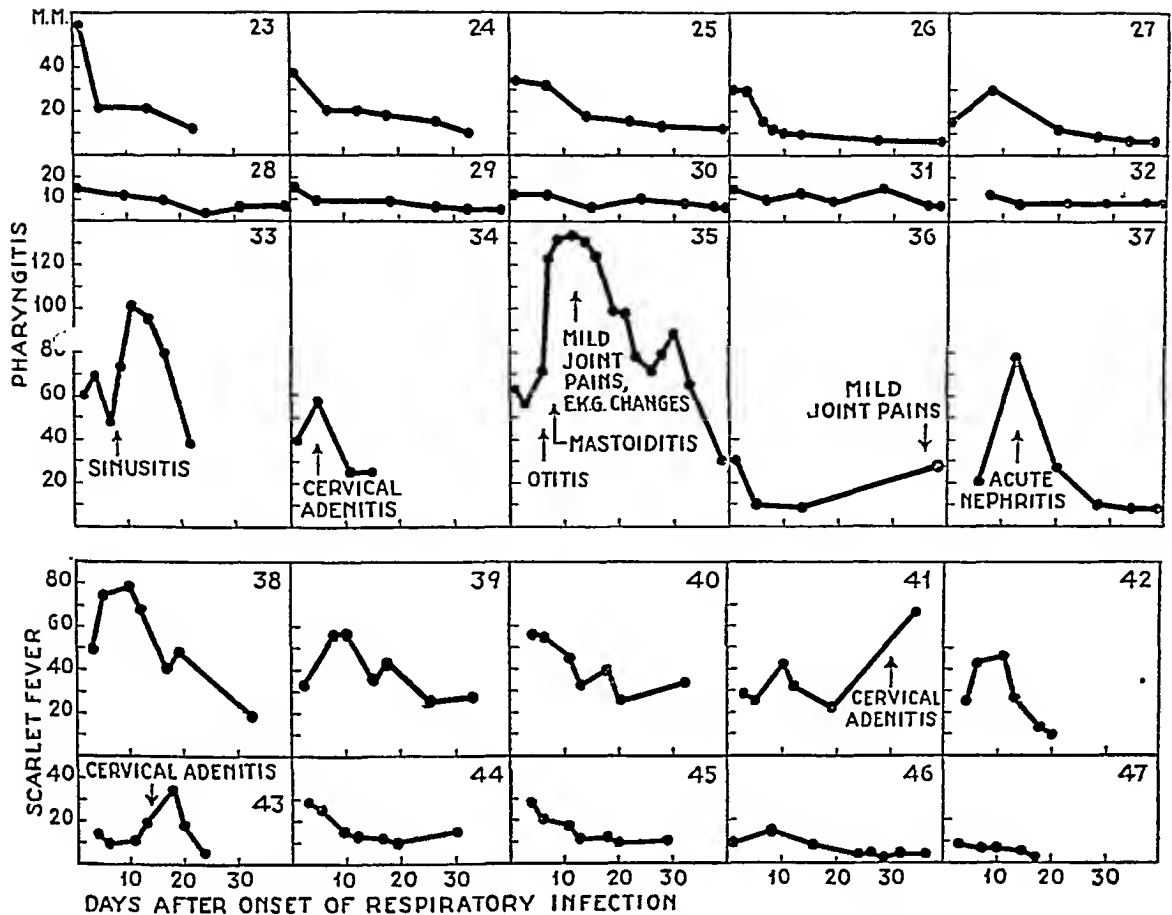


FIG. 3. SEDIMENTATION RATES OF CONTROL SUBJECTS WITH HEMOLYTIC STREPTOCOCCUS RESPIRATORY INFECTIONS—PHARYNGITIS AND SCARLET FEVER.

graphic changes. In this case (Curve 35) the sedimentation rate rose to a level as high as that which usually accompanies rheumatic carditis (cf. Figure 2). Similar observations have been reported by Rhodin (13) who found a moderate increase in sedimentation rate during scarlet fever followed by a second larger increase in the event of complications, with a well marked minimum between them. The curves of several patients whose sedimentation rates were determined at frequent intervals showed cyclic fluctuations during the first week or two after infection similar to those observed by Rhodin (13) in scarlet fever. In our series of patients, this type of curve occurred not only during convalescence from scarlet fever (Patients 38, 39, 40, 41) but also during Phase II of acute rheumatism (Patients 13, 21). In summary, increases in sedimentation rate

may or may not occur during initial hemolytic streptococcus pharyngitis. It does occur during septic complications and during sequelae characterized by sterile inflammation.

#### *Plasma proteins in relation to the erythrocyte sedimentation rate*

The literature dealing with the factors in blood responsible for an increased sedimentation rate contains a wide variety of conclusions. However, the majority of investigators are agreed that the decisive factors reside in the plasma or serum rather than in the cells, and that abnormal sedimentation is accompanied by quantitative alterations in the protein fractions of plasma. Fåhræus (14) pointed out that the amount of fibrinogen or serum globulin paralleled the sedimentation rate. This has been confirmed by Westergren et al.

TABLE I

*Serum protein values of nine patients with acute rheumatism, and of nine healthy control subjects*

Patients with acute rheumatism				Controls			
Total serum protein	Serum albumin	Serum globulin	A/G ratio	Total serum protein	Serum albumin	Serum globulin	A/G ratio
<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>		<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>	
6.8	3.9	2.9	1.34	6.7	4.6	2.1	2.19
7.4	4.2	3.1	1.35	7.3	4.8	2.5	1.92
7.0	4.5	2.5	1.82	6.9	4.6	2.3	2.00
7.2	4.8	2.5	1.92	7.2	4.9	2.3	2.13
8.1	4.7	3.4	1.40	7.2	4.8	2.4	2.00
8.8	4.7	4.1	1.14	7.3	4.9	2.4	2.04
8.0	4.1	3.9	1.05	7.0	4.7	2.3	2.04
8.2	4.5	3.7	1.16	7.1	5.2	1.9	2.74
7.9	4.5	3.4	1.32	7.3	5.1	2.2	2.32
Average 7.7	4.4	3.3	1.39	7.1	4.8	2.3	2.15

(15) and by Bendien and Snapper (16) for a number of miscellaneous diseases. Gilligan and Ernstene (17) found a striking correlation between the quantity of plasma fibrinogen and the sedimentation rate in rheumatic fever. High levels of fibrinogen seem to be the rule in this disease, and we have also observed high levels of serum globulin in a series of nine patients with acute rheumatism accompanied by sedimentation rates of more than 100 mm. in 1 hour (See Table I). However, the fact that high protein values accompany high sedimentation rates in no way proves a causal relationship.

In order to determine whether one of these factors was actually the cause of the high sedimentation rates in rheumatic fever, we attempted to reproduce the conditions necessary for rapid sedimentation by the modification of normal plasma (or serum) in various ways, including the addition of protein fractions isolated from the blood of normal individuals and patients with rheumatism. The sedimentation rates of washed human erythrocytes resuspended in modified plasma were then measured under standard conditions, in comparison with parallel measurements taken on red cells resuspended in the unmodified plasma of normal individuals and rheumatic patients.

The following technical precautions were observed throughout:

1. All the cells and sera used in any one experiment were of the same blood group.
2. Erythrocytes were washed three times with isotonic

NaCl, kept in the refrigerator, and used only if less than 48 hours old.

3. Isotonicity was assured by dialyzing all protein fractions overnight against 0.85 per cent NaCl.

4. Sodium citrate was used as anticoagulant.

5. Long glass tubes (500 mm.) were used for sedimentation, to minimize the effect of packing.

Protein fractions were prepared as follows. Fibrinogen was precipitated from whole citrated plasma by the addition of an equal volume of saturated NaCl. This precipitate was dissolved in water and dialyzed, first against distilled water to remove excess salt, then against isotonic saline, and finally against isotonic saline under slight vacuum to reduce the volume to the desired level. The dialysis procedure was the same for all fractions.

Plasma globulin was precipitated from the supernatant fluid, after the removal of fibrinogen, by full saturation with NaCl. The precipitate was dissolved in distilled water and dialyzed.

Serum globulin was precipitated from serum by saturation with NaCl, dissolved in water and dialyzed. In rapidly sedimenting blood this fraction included a small amount of "residual" fibrinogen; i.e., a protein left in solution after clotting was complete, which could be precipitated by one-half saturated NaCl.

Globulins were not subjected to further fractionation.

The albumin fraction included all protein left in the supernatant after full saturation with NaCl. Dialysis was performed as usual.

Certain factors were found to play no significant part in the sedimentation mechanism. (1) *Total serum lipoids*: Serum defatted by Hartley's (18) method produced a sedimentation rate equal to that of untreated serum, in the case of both a slowly and a rapidly sedimenting blood. Similar observations have been recorded by Theorell (19)

and by Ohlson and Rundquist (20). (2) *Serum complement*: Inactivation of serum by heating at 56° for 30 minutes produced only slight changes in sedimentation rate.

Serum of rheumatic patient ..... 80 mm.  
Inactivated serum of same patient ..... 75 mm.

(3) *Plasma crystalloids*: Dialysis of whole plasma or whole serum did not affect the sedimentation rate provided that isotonicity was restored and that the concentration of total protein was not changed.

Certain factors which have been shown by previous workers to affect the sedimentation of erythrocytes were investigated in "reconstructed" bloods (plasma or serum plus washed cells), and were found to operate in the usual way. The sedimentation rate was slowed by increasing the ratio of red cells to plasma, and vice versa. Reduction of the total volume of normal plasma without increasing the salt concentration (by dialysis under negative pressure against isotonic saline), also diminished the sedimentation rate.

Whole plasma ..... 45 mm.  
Same, reduced to  $\frac{4}{5}$  of original volume, isotonicity maintained ..... 30 mm.

It was also found that dilution of plasma with isotonic salt (or Ringer's solution) resulted in a marked retardation of the sedimentation rate. Dilution of plasma to one-half or one-third of its original concentration resulted in a sedimentation rate approximately equal to that of the diluent alone. This is illustrated in Table II. The sedimentation rates are expressed as millimeters in 30 minutes.

The removal of fibrinogen from the plasma obtained from normal or rheumatic patients slowed sedimentation considerably; nevertheless, sera

from rheumatic patients showed evidence of a residual factor of some magnitude.

	Plasma mm.	Serum mm.
Rheumatic patient (a) .....	110	85
Rheumatic patient (b) .....	120	45
Rheumatic patient (c) .....	100	50
Normal individual <sup>2</sup> (a) .....	10	4
Normal individual <sup>2</sup> (b) .....	55	20

Albumin (i.e., serum from which the globulin had been removed) inhibited sedimentation almost completely.

#### Sample A

Plasma .....	135 mm.
Serum .....	63 mm.
Serum minus globulin .....	8 mm.

#### Sample B

Plasma .....	100 mm.
Plasma minus fibrinogen and globulin .....	9 mm.

It was not clear whether the disappearance of the sedimentation factor from serum on removal of globulin was due to the absence of globulin per se, or whether the inhibitory effect of dilution with saline was coming into play. This point was investigated further by incorporating fibrinogen or globulin fractions, or both, into equivalent quantities of normal plasma. As the final volume of the modified plasma was the same as that of the original normal plasma, the protein concentration of the modified plasma was higher than before. If the fractions used for modification had been inactive, their addition to normal plasma should have inhibited sedimentation, owing to the increase in the concentration of total protein. But the sedimentation rate of modified plasma was in every case higher than that of normal<sup>2</sup> plasma. The observed effects of adding or removing globulins must therefore be related to the presence or absence of these particular proteins, and not merely to changes in the relative amounts of total protein and salt. Typical experiments are presented in Table III.

TABLE II

*The effect on the sedimentation rate of diluting plasma with physiological salt solutions*

Plasma, cc. ....	1.0	.8	.6	.4	.2	0
Diluent, cc. ....	0	.2	.4	.6	.8	1.0
NaCl, 0.85 per cent. ....	mm.	mm.	mm.	mm.	mm.	mm.
Ringer's solution .....	120	95	60	3	0	3
		130	50	15	10	2
NaCl, 0.85 per cent. ....	135	120	55	25	4	4
NaCl, 0.85 per cent. ....		70	25	8	2	6
Ringer's solution .....		70	30	4	2	1

<sup>2</sup> The variability of the readings recorded for unmodified normal plasma is due to variations in the density of different lots of cell suspensions used for the tests. The sedimentation rates of all normal blood samples used, as determined by the Westergren technique, were less than 20 mm. in 1 hour.

TABLE III

*The effect on the sedimentation rate of modifying normal plasma by the addition of various protein fractions from normal and rheumatic blood*

Experiment 1	
	mm.
Normal plasma .....	55
Normal plasma plus fibrinogen and globulin from an equal volume of normal plasma .....	95
Experiment 2	
Normal plasma .....	35
Normal plasma plus fibrinogen from equal volume of rheumatic plasma .....	82
Experiment 3	
Normal plasma .....	17
Normal plasma plus fibrinogen from rheumatic plasma .....	43
Normal plasma plus globulin from rheumatic plasma .....	30
Experiment 4	
Normal plasma .....	5
Normal plasma plus fibrinogen from rheumatic plasma .....	60
Normal plasma plus globulin from rheumatic plasma .....	12
Plasma from rheumatic patient .....	140

Fibrinogen was definitely more effective than globulin in every case studied; nevertheless the globulin fractions showed considerable activity. However, the sedimentation rates of modified normal plasmas were in no case as high as those of the rheumatic plasmas from which the various fractions had been prepared. This may be attributed to partial denaturation, especially of fibrinogen, incident to precipitation, dialysis and other handling. In this connection, it may be of interest to note that the activity of serum separated from clotted blood was slightly higher than that of serum obtained by defibrination.

Similar experiments have been reported by Zárday and Farkas (21), who modified normal whole blood by the addition of graded amounts of normal fibrinogen, globulin and albumin. They found that the sedimentation rate was increased in proportion to the amount of fibrinogen and globulin added. Extra albumin, in contrast, reduced the rate. Our findings in acute rheumatism are entirely in accord with those of Zárday and Farkas for normal blood. It is therefore concluded that the interpretation of Fåhræus (14), that changes in sedimentation rate depend on changes in the fibrinogen and globulin fractions of plasma, is applicable to acute rheumatism.

The mechanism whereby these proteins accelerate sedimentation is a matter of much contro-

versy, and need not concern us here. A full discussion of the subject with complete bibliography is to be found in a recent monograph by Reichel (22).

*Immunological studies on fibrinogen and globulin from rheumatic patients*

The experiments just described indicated quantitative differences between the major protein fractions of normal (slowly sedimenting) and rheumatic (rapidly sedimenting) bloods. They did not show whether there might be qualitative differences. In order to investigate this point, an experiment was set up to detect immunological specificity of fibrinogen and globulin in rheumatic versus normal individuals, by means of precipitin tests with antisera to these proteins before and after absorption with homologous and heterologous protein fractions.

Fibrinogen and globulin fractions were obtained from the plasma of two classes of subjects: (1) Patients with acute rheumatism, with sedimentation rates of more than 100 mm. in 1 hour; and (2) normal, healthy individuals with normal sedimentation rates. The protein solutions were all adjusted to contain equal concentrations of nitrogen. A portion of each sample was used for immunizing two rabbits, the remainder was kept sterile in the ice box. Strong precipitating antisera were obtained after four weeks of immunization. These antisera were divided into three parts. One part was stored untreated. One part was absorbed with its homologous antigen. The third part was absorbed with the corresponding protein fraction from the other class of subject. Absorptions were performed at 37° C. After centrifugation, precipitin tests were set up with the supernatant serum.

These tests showed no distinction between normal and rheumatic fibrinogen or between normal and rheumatic globulin. It was not possible to absorb the antiserum to a fraction from a rheumatic patient with its normal equivalent and obtain a supernatant fluid which would give a precipitin reaction only with the homologous antigen. If absorption was complete, the serum could no longer be precipitated by either type. If incomplete, the serum could be precipitated equally well by protein from both normal and rheumatic individuals. Sample protocols are presented in

TABLE IV

*Precipitin reactions between plasma protein fractions and their antisera, before and after absorption\**

Antigen globulin	Rabbit antiserum to rheumatic globulin D, undiluted											
	Unabsorbed						Absorbed with					
							Rheumatic globulin D			Normal globulin B		
Rheumatic D	++	++	++	(++++)			0	+	+	(++)		
Normal B	+	++	++	(+++)			0	0	±	(++)		
Antigen fibrinogen	Rabbit antiserum to rheumatic fibrinogen Be, undiluted											
	Unabsorbed						Absorbed with					
							Rheumatic fibrinogen Be			Normal fibrinogen B		
Rheumatic Be	+++	+++	+++±	(++++)			+	+	±	(++±)		
Normal B	++	++	++	(+++)			+	+	±	(+++±)		

\* Four readings are given for each test: (1) after 20 minutes at room temperature; (2) after 2 hours' incubation at 37.5° C.; (3) after 18 hours in the refrigerator; (4) after centrifugation.

Table IV. A qualitative change in the fibrinogen and globulin fractions of the blood in acute rheumatism was not demonstrable by this method. The possibility of detecting such a change by more refined procedures remains open.

#### DISCUSSION

Having explained the high sedimentation rates of acute rheumatism as the result of increased plasma fibrinogen and globulin, our next problem is to account for the change in the proteins. At this point direct evidence comes to an end, and we can only look to the work of other investigators for possible analogies.

There is some evidence to indicate that fibrinogen and globulin are produced by the reticulo-endothelial system. For example, sharp, transient increases in fibrinogen occur within two hours after the injection of substances which are taken up by reticulo-endothelium, according to Held and Behr (23), whereas "blocking" of the reticulo-endothelial system by the previous injection of colloidal copper prevents this response. From these and other experiments it seems that an increase of fibrinogen may be a direct response of reticulo-endothelial cells to stimulation.

Increases in sedimentation rate during and following hemolytic streptococcus respiratory infections occur under three different clinical conditions. First, during the initial infection there may

be a mild rise in sedimentation rate which probably represents the response of reticulo-endothelium to foreign substances in general. Second, during septic complications there is a further increase in sedimentation rate which may occur in response to further invasion of the host by the infectious agent. The third condition is fulfilled by sterile inflammatory processes such as acute rheumatism, the onset of which is accompanied by a steep rise in the sedimentation rate curve to a high level. The intensity of this response suggests that a mechanism may be involved which differs from that in the first two conditions.

A number of observations have been made by independent authors which may apply to the development of the high sedimentation rate in acute rheumatism. One of these is Berger's (24) finding, that the second of two equal injections of foreign protein is followed by a much larger increase in serum globulin than the first, when the second dose is given after the complete subsidence of the globulin response to the first. The globulin curve following widely separated injections of foreign protein is similar to the sedimentation rate curve of pharyngitis followed by acute rheumatism.

Another possible analogy which presents itself is the development of high sedimentation rates by tuberculin-sensitive patients (15) or tuberculous rabbits (25) in response to injections of old tu-



berculin. In these instances, as in rheumatism, the inflammatory process appears to be sterile and the sedimentation rate rises rapidly to high levels. Normal controls show no rise in sedimentation rate when similarly injected. Roch (26) has recently described a similar response following repeated injections of swine serum into rabbits. In his series, increases of sedimentation rate were largely confined to skin-sensitive animals (Arthus phenomenon).

The above findings all indicate that sharp increases of sedimentation rate can be expected in response to repeated doses of foreign protein. The rheumatic subject necessarily receives a dose of foreign protein during acute pharyngitis. A second dose of the same foreign protein received at the end of Phase II would account for the observed increase in sedimentation rate at that time. Whether there is a second dose, and if so, the nature of the protein involved, remain to be established.

#### SUMMARY

In acute rheumatism, the sedimentation rate may be considered as a measure of the extent of inflammation.

The increased sedimentation rate in acute rheumatism is caused by an increase in plasma fibrinogen and globulin.

An immunological test for a qualitative difference between the plasma protein fractions of normal and rheumatic individuals gave negative results.

A possible type of mechanism is suggested to account for the rise in sedimentation rate just before the onset of a rheumatic attack.

The authors are indebted to Dr. A. B. Gutman and Ethel B. Gutman for a number of estimations of serum protein.

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